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UNIVERSITY OF ALBERTA

THE EFFECTS OF DIETARY OMEGA-3 FATTY ACIDS ON MEMBRANE COMPOSITION AND INSULIN RESPONSIVENESS IN RAT ADIPOCYTES

BY

ARLENE M. PARROTT

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

IN

NUTRITION

DEPARTMENT OF FOODS AND NUTRITION

EDMONTON, ALBERTA

SPRING 1992

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UNIVERSITY OF ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **"The effects of dietary omega-3 fatty acids on membrane composition and insulin responsiveness in rat adipocytes"** submitted by **Arlene M. Parrott** in partial fulfillment of the requirements for the degree of **Master of Science in Nutrition**.

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Dr. J. E. E. Van Aerde

Date: December 20 1991

ABSTRACT

The relationship among diet fat-induced alterations in plasma membrane phospholipids, insulin binding and glucose transport in isolated adipocytes of control and streptozotocin-induced diabetic rats was assessed. Animals were fed nutritionally complete semi-synthetic diets differing in the content of long-chain ω-3 fatty acids while maintaining a constant ω-6 fatty acid level. The balance between dietary saturated fatty acids and ω-6 fatty acids is an important factor determining the efficacy of ω-3 fatty acid induced alterations in biochemical parameters. Therefore, the effect of dietary ω-3 fatty acid level was examined in animals fed diets with high or low polyunsaturated to saturated (P/S) fatty acid ratios.

Increasing the ω-3 fatty acid content of the diet increased the ω-3 fatty acid content while decreasing the arachidonic acid content of the major plasma membrane phospholipid fractions. These changes were most pronounced on a low P/S diet. The overall polyunsaturated content of membrane phospholipid fractions was not altered by dietary ω-3 fatty acid supplementation. Altering the ω-3 fatty acid content of the diet did not significantly affect insulin binding or glucose transport in adipocytes, suggesting that the amount of polyunsaturated fatty acids present in the membrane, regardless of ω-6 or ω-3 fatty acid type, is a more important determinant of these two membrane-mediated functions.

This thesis demonstrates that moderate dietary supplementation of ω-3 fatty acids did not diminish peripheral glucose utilization in adipose tissue in this animal model.

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I. REGULATION OF INSULIN BINDING AND ACTION

Insulin is an anabolic hormone with effects upon carbohydrate, lipid and protein metabolism. The classical target tissues for insulin action include muscle, liver, and adipose tissues. However, insulin receptors have been identified in almost all mammalian cells (Rosen,1987). In target cells, insulin action includes the promotion of glucose and amino acid uptake, lipogenesis, glucose oxidation and the synthesis of protein and glycogen. Many of these effects are tissue specific and the chronology of insulin action varies. For example, while glucose transport is stimulated within seconds of the addition of insulin to cells, the synthesis of protein, lipid and nucleic acid requires several hours of insulin exposure (Kahn, 1985).

In this chapter, the regulation and factors affecting insulin binding and action and the pathogenesis of insulin resistant states will be reviewed.

A. INSULIN RECEPTOR

Structure

Like other peptide hormones, insulin action is initiated by the binding of insulin to a specific plasma membrane receptor. The insulin receptor is composed of two distinct subunits derived from a single polypeptide precursor. The α-subunit has a molecular weight of 135,000 Daltons and is believed to contain the binding site for insulin. As this subunit is sensitive to proteolytic cleavage, it is believed to have an

extracelluar domain (Kasuga et al.,1982). The β-subunit is a transmembrane protein which has a molecular weight of 95,000 Daltons. The β-subunit displays a tyrosine specific protein kinase activity and autophosphorylation of tyrosine residues (Roth & Cassell,1982). This suggests that the β-subunit is involved in signal transduction across the membrane (Kahn,1985).

The insulin receptor is usually found as a tetramer in which two pairs of disulphide-bonded α-β dimers are joined by disulphide bonds between their α chains. Differences in the nature of the disulphide bonding have also been suggested (Yip & Moule,1983). Both receptor subunits are glycosylated but the functional significance of the carbohydrate moieties are not known. However, removal of the carbohydrate chains decreases insulin binding (Jacob & Cuatrecasas,1981).

Binding

Binding of insulin to its cell-surface receptor is the initial step in insulin action. Insulin-receptor binding is generally measured <u>in vitro</u> by interaction of radio-labelled hormone with the receptor on whole cells or in membrane-rich cellular fractions. Insulin-receptor binding has been demonstrated to be rapid, reversible and site specific for insulin (Pederson,1984). It should be pointed out that cells contain "spare receptors", therefore, maximal responsiveness to insulin can be achieved before all receptors are occupied by insulin (Pedersen,1984). The number of spare receptors varies widely between cell types. Studies indicate that maximal responsiveness of adipocytes can be achieved when approximately 10% of the receptor complement is occupied by insulin while

maximal responsiveness in hepatocytes is only achieved when approximately 90% of the receptors are occupied (Gammeltoft,1984). This difference in receptor occupancy allows for hepatocytes to be more sensitive to changes in insulin concentration.

Scatchard analysis of insulin binding data generally produces a curvilinear Scatchard plot. A nonlinear plot may be due to the presence of two or more classes of independent binding sites with different receptor affinity and receptor concentrations or to a single class of receptors with negative cooperative interactions. Interpretation of insulin binding data has not been unequivocally accepted but insulin binding is generally thought to be of the negative cooperative type. Physiologically, as insulin action is proportional to receptor occupancy, negative cooperativity would maintain insulin sensitivity at low insulin concentration but attenuate the cellular response at higher insulin concentrations (Gammeltoft,1984).

The half-life of the receptor is estimated to be between 7 and 12 hours (Kahn,1985). While information is limited on the biological fate and regulatory mechanisms involved in metabolism of the insulin receptor, receptor degradation may result through the process of receptor-mediated endocytosis. Insulin-receptor degradation appears to occur in lysosomes, however, some receptors escape degradation and are recycled back to the plasma membrane (Fehlman et al.,1982). The half-life of the receptor is shortened by exposure of the cell to insulin. Down regulation of receptors may play a role in enabling cells to regulate their sensitivity to ambient concentrations of insulin (Bar et al.,1976).

B. TRANSMEMBRANE SIGNALLING

Insulin Receptor Tyrosine Kinase Activity

Binding of insulin to its cell-surface receptor mediates one or more signalling mechanisms resulting in the biological actions of insulin. However, the details of these proposed molecular signals are not fully understood. Kasuga et al.(1982a) reported in intact cells that insulin-stimulated phosphorylation of the receptor β-subunit occurred on seryl, threonyl and tyrosyl residues. In cell-free systems, insulin binding has been shown to result in phosphorylation of tyrosine residues exclusively (Kasuaga al.,1982b). Subsequent studies have identified the insulin receptor as a protein tyrosine kinase capable of the autophosphorylation of tyrosine residues on the β-subunit. Unoccupied insulin receptors incubated with auto-receptor antibodies were shown to immunoprecipitate both the receptor and the receptor kinase (Zick et al.,1983). Additionally, Kasuga et al.(1983) demonstrated that highly purified receptors still retain the kinase activity. Affinity labelling with ATP analogues and information gained from cloning of the insulin receptor indicate that the β-subunit of the receptor contains an ATP-binding site characteristic of protein kinases (Roth & Cassell,1982). The phosphorylation of serine and threonine residues of the receptor appears to occur via other kinases in the cell (Rosen,1987). These observations provide support for the identification of the insulin receptor as a protein tyrosine kinase. This property is also shared by plasma membrane receptors for polypeptide ligands such as epidermal growth factor,

platelet derived growth factor and insulin-like growth factor-1 (Kahn,1985).

Using a partially purified receptor immobilized on an insulin-agarose column, Yu and Czech (1984) examined regulation of insulin receptor kinase activity by phosphorylation and dephosphorylation of amino acid residues. Phosphorylation of the insulin receptor with increasing concentrations of ATP resulted in increased activation of receptor kinase activity. Conversely, incubation of phosphorylated insulin receptor with alkaline phosphatase resulted in selective dephosphorylation of phosphotyrosine residues on the β-subunit. Dephosphorylation of the receptor caused a significant inhibition of the receptor kinase activity. Recently, Tornqvist and Avrich (1988) have shown that the dephosphorylated receptor is virtually inactive as a protein kinase.

In contrast to tyrosyl phosphorylation, seryl and threonyl phosphorylation of the receptor β-subunit has been shown to decrease receptor kinase activity, rendering the receptor less susceptible to activation by autophosphorylation (Stradtmauer & Rosen,1986). Thus it is possible that tyrosine phosphorylation activates the insulin receptor kinase and seryl-threonyl phosphorylation reverses the activation, resulting in inhibition of the kinase (Rosen,1987).

Subsequent studies by Tornqvist & Avruch (1988) indicated tyr-1146, 1150 and 1151 are most closely associated with kinase activation. While phosphorylation of tyr-1316 and 1322 occurred during insulin-stimulated autophosphorylation, these residues could be involved in a function of the receptor that is distinct from tyrosine kinase activity.

The complementary DNA for human insulin proreceptor has been cloned and its primary amino acid sequence determined (Ullrich et al.,1985). The insulin proreceptor is a single gene, containing a protease-sensitive region where cleavage gives rise to α and β subunits. The receptor α-subunit contains a cysteine-rich domain and does not contain any long hydrophobic regions by which it may be anchored to the membrane. Thus, as predicted, the α-subunit probably has an exclusive extracelluar domain (Ullrich et al.,1985). The receptor β-subunit contains a 23-amino acid long transmembrane domain which is predicted to traverse the membrane once. The cytoplasmic kinase domain of the β-subunit shows sequence similarity to the src family of oncogene products and human epidermal growth factor receptor (Ullrich et al.,1985).

Precise mechanisms involved in activation following the binding of insulin to the receptor α-subunit have not been clearly identified. Heffertz & Zick (1986) have suggested that receptor cross-linking or aggregation, resulting in alteration of the conformation of the β-subunit, is required for kinase activation. Additionally, the transfer of the insulin binding signal to the kinase domain may require the association of two intact receptor halves (i.e. two α-β dimers)(Boni-Schnetzler et al.,1987).

Research indicates that protein kinase activity of the insulin receptor is necessary for insulin action. Chou et al. (1987) developed Chinese hamster ovary cell lines that expressed either a normal insulin receptor or a receptor which substituted alanine for lysine-1018 in the ATP-binding domain of the β-subunit. The mutated receptors were shown to bind insulin but did not possess kinase activity. In contrast to kinase-

deficient receptors, normal human insulin receptors were able to mediate insulin-dependent deoxyglucose uptake, S6 kinase activation, endogenous substrate phosphorylation, glycogen synthesis and thymidine incorporation into DNA. Similar studies have been performed using human insulin receptors incorporated into Chinese hamster ovary cell lines (McClain et al.,1987; Hari & Roth,1987). Mutated receptors lacking kinase activity had either lysine-1018 replaced by alanine (McClain et al.,1987) or arginine-1030 replaced by lysine (Hari & Roth,1987) in the ATP-binding domain. In both cases, insulin was found to be incapable of internalizing the mutated receptors. These studies indicate that the tyrosine kinase activity of the insulin receptor is important in mediating several insulin-activated effects.

Intracellular Mediators

Identification of biochemical mechanisms responsible for relay of initial insulin-induced messages to cytosolic enzymes has been the subject of recent investigations. Czech et al. (1988) hypothesize that a phosphorylation cascade initiated by the tyrosine phosphorylation of serine/threonine kinases or phosphatases couples the activated insulin receptor kinase to insulin action. Further research is required to define a biologically relevant substrate which could initiate a phosphorylation cascade.

A second theory suggests that insulin action may involve intracellular generation of low molecular weight compounds that modulate activity of key enzymes (Low & Saltiel,1988). According to this hypothesis, the insulin receptor controls activation of a specific

phospholipase C leading to cleavage of phosphatidylinositol-glycans (PI-glycans) from the cell membrane. This cleavage produces two potential second messengers, inositol-glycan compounds and a unique species of 1,2-diacylglycerol. The inositol-glycans may act through serine protein kinase and phosphatases resulting in specific enzyme activation while the diacylglycerol may activiate protein kinase C..

Saltiel et al.(1986) first reported rapid insulin-stimulated hydrolysis of a membrane phosphatidylinositol-glycan that resulted in production of two potential second messengers, however, similar results have also been reported from other laboratories (Farese et al.,1988). The chemical structure of the inositol-glycans has not been completely elucidated. However, insulin-sensitive [^{3}H]-inositol and [^{3}H]-glucosamine-labelling as well as nitrous acid sensitivity, confirms the presence of an inositol monophosphate linked to non-acetylated glucosamine (Saltiel et al.,1987). The composition of the remaining monosaccharides has not been determined, however, labelling studies indicate the presence of at least one mannose residue (Low & Saltiel,1988). The structure of these PI-glycan compounds may potentiate insulin activity in cellular metabolism. As well, [^{3}H]myristate-labelling indicates that myristate-diacylglycerol is produced by phosphatidylinositol-glycan cleavage (Saltiel et al.,1986). Phosphatidylinositol and its phosphorylated species are well-defined intracellular mediators (in other cell types). Previous studies have shown that dietary ω-3 fatty acid supplementation can alter the fatty acid composition of membrane phosphatidylinositol. These changes in phosphatidylinositol fatty acid composition have been shown to influence cellular functioning (Holub,1985).

In vitro, PI-glycans appear to mimic the effects of insulin in a number of enzyme systems. PI-glycans, isolated from liver membranes after treatment with insulin or with a glycosated-phosphatidylinositol-specific phospholipase C from Staphylococcus aureus, have been shown to modulate activities of cAMP-phosphodiesterase (Saltiel et al.,1986; Saltiel,1987), adenylate cyclase (Saltiel,1987), pyruvate dehydrogenase (Saltiel,1987) and phospholipid methyltransferase (Kelly et al.,1987). Inositol-glycans also result in phosphorylation and dephosphorylation of cytosolic proteins in a manner similar to insulin. For example, in a cell-free system utilizing radiolabelled ATP, inositol-glycans stimulate phosphorylation of several cellular proteins including ATP citrate lyase and the ribosomal protein, S6. The proteins phosphorylated by inositol-glycans correspond to proteins phosphorylated by insulin in intact cells (Alemany et al.,1987). Additionally, preparations of inositol-glycans have been demonstrated to cause the direct inhibition of insulin release in isolated rat islet cells (Malaisse et al.,1989). These results provide evidence that inositol-glycans may stimulate the same signalling pathways as insulin. In contrast, inositol-glycans have been demonstrated to have no effect upon glucose transport in intact adipocytes (Kelly et al.,1987). Thus, the inositol-glycan hypothesis provides a possible, but only partial explanation for insulin-mediated responses of the cell.

Expansion of the inositol-glycan hypothesis includes suggestions as to the specific mechanisms which may be involved in insulin-regulated cleavage of phosphatidylinositol-glycans. The hydrolysis of phosphatidylinositol-glycans has been shown to be stimulated by insulin (Saltiel et al.,1986; Saltiel et al.,1987, Saltiel, 1987). However, this

action can also be produced by a distinct and specific phosphatidylinositol phospholipase C (Saltiel,1987). Fox et al.(1987) have purified a phospholipase C from rat liver plasma membranes which is specific for glycosylated-phosphatidylinositols; no hydrolysis of phosphatidylinositol, phosphatidylinositol 4,5-biphohphate or phosphatidylcholine was observed. Further research will indicate whether this phospholipase C is an effector for some of the metabolic actions of insulin.

A role for a G-protein as an intermediate in coupling insulin-receptor activation to phospholipase C has been hypothesized (O'Brien et al.,1987). Support for this mechanism comes from O'Brien et al.(1987) who found that guanine nucleotide-regulatory proteins, G_0 and G_i, could be phosphorylated by the insulin receptor tyrosine kinase. Pre-treatment of BC3H-1 cells with pertussis toxin, an inhibitor of G-proteins, has been reported to completely block insulin-stimulated generation of diacylglycerols (Luttrell & Rogel,1988). As G-proteins are embedded within the lipid bilayer of the plasma membrane, alterations in fatty membrane composition due to changes in diet fat composition may influence G-protein functions.

Studies have indicated that protein kinase C can mimic certain actions of insulin as well as inhibiting other insulin-stimulated actions (Saltiel & Cuatrecasas,1988). Thus, the role of diacylglycerols in insulin action is contentious. Saltiel & Cuatrecasas (1988) have suggested that insulin may cause a selective activation of protein kinase C due to the structurally distinct diacylglycerols produced by phosphatidylinositol-glycan cleavage. This possible mechanism of action

and possible influence of diet fat altercations in the fatty acid composition of the diacylglycerol requires further investigation.

In summary, possible second messengers for insulin have been identified which mimic specific actions of insulin. However, it is still premature to regard these compounds as second messengers for any of the actions of insulin (Saltiel,1991). These mediators appear to be generated by the hydrolysis of phosphatidylinositol-glycans in the plasma membrane by a specific phospholipase C. This reaction produces two potential second messengers, inositol-glycan compounds and a distinct diacylglycerol, which may regulate certain insulin effects. As these events occur within the lipid bilayer of the plasma membrane, changes in diet fat which alter the phospholipid fatty acid composition may also influence these processes. However, the inositol-glycan hypothesis provides only a partial explanation for insulin-mediated actions on the cell. This suggests that other mechanisms of insulin action may also exist. Further research is necessary to determine the precise chemical structures and biological activities of these compounds to define their role in mediating insulin action. Clearly, as insulin action involves a cascade of events, abnormalities anywhere along this sequence may lead to insulin resistance.

C. INSULIN ACTION

Glucose Transport

Hexose uptake by cells is brought about by transport across the plasma membrane through membrane-bound proteins, glucose "carriers". In

muscle and fat cells, glucose uptake is stimulated by insulin and occurs within seconds of exposure to the hormone (Klip,1982). This process of glucose transport is tightly regulated and has been extensively studied. Many studies have utilized isolated adipocytes because of the large effect of insulin, the homogeneity of the cell preparation and the relative accessibility of the tissue samples (Gliemann & Rees,1983).

Recent cDNA cloning studies have found that glucose transport proteins comprise a family of structurally related proteins encoded by distinct genes that are expressed in a tissue-specific manner (Kahn & Flier,1990). Currently, five distinct glucose transporters have been identified.

Fukumoto et al. (1989) have proposed nomenclature (GLUT1 through GLUT5) which refers to the transporters in the order in which they were cloned. GLUT1 is widely expressed in tissues independent of whether glucose transport is rapidly stimulated by insulin. GLUT2 is found only in liver, kidney, basolateral membrane of intestinal cells and pancreatic beta-cells. In these cell types, except for pancreatic beta-cells, glucose release into the bloodstream can occur. GLUT3 is also expressed in many tissues; however, little is known regarding its regulation. GLUT4 is present in white and brown adipose tissue and muscle tissue. These tissues are highly insulin-sensitive and glucose transport increases rapidly in response to insulin exposure. GLUT5 has been located in small intestine, kidney, skeletal muscle and adipose tissues; however, its regulation has not yet been determined (Kahn & Flier, 1990).

Concurrent work by Cushman & Wardazala (1980) and Suzuki & Kono (1980) initially proposed that adipocyte glucose transporters are

sequestered in intracellular membrane pools which are translocated to the plasma membrane upon exposure of the cell to insulin. This translocation is rapid, reversible and insulin concentration dependent (Karnieli et al.,1981).

Recent work has indicated that at least two of the glucose transporters, GLUT1 and GLUT4, probably reside in different intracellular vesicles (Zorzano et al.,1989). While both of these glucose transporters can be translocated to the plasma membrane in response to insulin, evidence suggests that the magnitude of the response differs between the transporters in the adipocyte (Gould et al.,1989). In the basal state, most GLUT1 transporters are located in the plasma membrane while GLUT4 transporters are located within intracellular vesicles. With insulin exposure, there is an approximate 10-fold increase in GLUT4 transporters present in the plasma membrane of the adipocyte but only a 2 fold increase in GLUT1 transporters (Kahn et al.,1989). Zorzano et al. (1989) have also shown that the total number of GLUT4 transporters present in adipose cells is significantly greater than the number of GLUT1 transporters. These observations have suggested that GLUT4 transporters are the primary insulin-responsive glucose transporters in adipose tissue whereas GLUT1 transporters are involved in basal glucose transport (Kahn & Flier, 1990).

As D-glucose is rapidly metabolized, it is difficult to measure its transport. However, several assay methods have been well established to measure transport of nonmetabolizable sugars or sugar analogs (Pedersen & Gliemann,1981). D-glucose, 2-deoxyglucose and 3-O-methylglucose display similar transport characteristics, suggesting that their transport systems are identical (Pedersen & Gliemann,1981). 2-deoxyglucose is rapidly

transported and phosphorylated by hexokinase but not further metabolized (Pedersen & Gliemann,1981). Initial uptake of 2-deoxyglucose is linear until intracellular concentration of 2-deoxyglucose prevents further phosphorylation and uptake decreases (Pedersen & Gliemann,1981). Another glucose analogue, 3-O-methylglucose, cannot be trapped by phosphorylation (Olefsky,1978). Transport of 3-O-methylglucose is rapid and curvilinear as it quickly congests the intracellular water space, requiring transport to be measured during the initial few seconds. Assay methods utilizing 3-O-methylglucose have been well described, allowing reliable measurement of glucose transport (Whitesell & Abumrad,1985; Pedersen & Gliemann,1981).

D. FACTORS AFFECTING INSULIN BINDING & ACTION

Diet

Energy

Diet is an important factor in the regulation of glucose metabolism as both quantity and composition of energy affect insulin binding and action.

Fasting has profound effects upon glucose metabolism. Fasting is characterized by diminished insulin action and may be considered as a state of insulin resistance (Olefsky, 1976). In normal (Olefsky,1976) and obese (Olefsky & Reaven, 1975) rat adipocytes, fasting results in an increase in insulin binding due to an increased binding affinity with no change in receptor number. Concurrently, there is a rapid, progressive and reversible decrease in the ability of rat adipocytes to transport glucose in response to insulin (Kahn et al.,1988). Similar results have been described in obese human adipocytes (Pedersen et al.,1982; Kolterman

et al., 1979a). Thus, in fasting a dissociation between changes in insulin receptor binding and insulin action is observed. In addition, refeeding after fasting has been demonstrated to not only restore insulin-stimulated glucose transport, but to transiently increase transport to supranormal levels (Kahn et al.,1988; Berger et al.,1989; Sivitz et al.,1989). The mechanisms for these changes appear to involve a depletion of GLUT4 transporters in the plasma membrane and intracellular pools during fasting (Kahn et al.,1988). GLUT1 transporter levels do not change significantly with fasting. With refeeding, the enhanced glucose transport in adipose cells is due to both increased GLUT4 number and an apparent increase in glucose transporter intrinsic activity (Kahn et al.,1988; Charron & Kahn,1990). The mechanism for alterations in the transporter intrinsic activity is unknown.

Carbohydrate/Fat

Numerous studies have indicated that high-fat, low-carbohydrate diets decrease glucose tolerance due to a change in the effectiveness of insulin (Olefsky & Saekow,1978; Ip et al.,1976; Begum et al.,1985; Salans et al.,1981; Lavau et al.,1979). However, the mechanisms by which high-fat diets cause insulin resistance are not fully understood. Several studies have reported reduced insulin binding to its receptor (Olefsky & Saekow,1978; Ip et al.,1976; Begum et al.,1985). It has been suggested that high-fat diets impair the ability to reinsert receptors into the plasma membrane following insulin induced internalization (Begum et al.,1985). However, this hypothesis is not supported by studies which

reported no change in receptor binding by feeding animals high-fat diets (Salans et al.,1981; Lavau et al.,1979).

In vitro studies employing adipocytes from animals fed high-fat diets have shown impaired insulin-mediated glucose transport, glucose oxidation, and lipogenesis (Olefsky & Saekow,1978; Ip et al.,1976; Salans et al.,1981; Lavau et al.,1979; Susini & Lavau,1978). Studies utilizing muscle tissue have also demonstrated reduced glucose transport, glucose oxidation, lipogenesis, glycolysis, and glucose incorporation into glycogen (Grundleger & Thenen,1982; Susini & Lavau,1978). Similarly, studies in man (Kolterman et al.,1979b) and animals (Susini & Lavau,1978) have demonstrated that high-fat diets as compared to high-carbohydrate diets significantly impair in vivo glucose utilization. Pedersen et al. (1991) have recently demonstrated a reduction in insulin-stimulated glucose transport in adipocytes from high-fat fed rats. This phenomenon is paralleled by a severe reduction in the number of GLUT4 transporters present in the cells. Since GLUT4 transporter number is decreased relatively more than the glucose transport rate, these researchers suggest that enhanced transporter intrinsic activity may compensate for the loss of transporters. The regulatory mechanisms for altered glucose transporter expression are unknown. However, Pedersen et al. (1991) suggest that the chronic insulinopenia accompanying the high-fat state as well as during energy restriction may be responsible. Feeding a high-carbohydrate diet to rats significantly reduced insulin binding while glucose transport (Grundleger & Thenen,1982; Olefsky & Saekow,1978), glucose oxidation and glucose incorporation into lipids (Olefsky & Saekow,1978) was significantly improved. In humans, high-carbohydrate

diets led to decreased insulin binding although *in vivo* insulin sensitivity was markedly enhanced (Kolterman et al.,1979b). Scatchard analysis suggests that short term changes in insulin binding are due to a reduction in receptor affinity while long term changes are a result of a reduction in the number of receptors (Kolterman et al.,1979b). It has been suggested that the contradiction of decreased insulin binding and increased insulin action caused by carbohydrate feeding may result from an increase in the activity of steps in glucose metabolism distal to the insulin receptor (Kolterman et al.,1979b).

Membrane Composition

Investigations indicate that dietary fat can influence the activity of hormone-stimulated and integral membrane protein functions. Membrane physical characteristics, determined by the fatty acid composition of polar lipids, polar head group components and cholesterol content can influence the biological function of membrane-bound enzymes (Clandinin et al.,1985). Alteration in type and amount of dietary fat has been shown to alter membrane phospholipid composition which coincides with change in mitochondrial ATPase activity (Innis & Clandinin,1981; Robblee & Clandinin,1984) and hormone-mediated functions such as liver plasma membrane glucagon-stimulated adenylate cyclase activity (Neelands & Clandinin,1983; Morson & Clandinin,1986). Similarly, as the insulin receptor is embedded within the lipid bilayer of the plasma membrane, diet induced change in plasma membrane phospholipid content and fatty acid composition influences activity of the insulin receptor in the adipocyte (Field et al.,1988).

A number of studies have indirectly suggested that membrane physical state may influence insulin binding and insulin receptor mediated responses. An increase in temperature is associated with an increase in number of available binding sites and enhancement of insulin stimulated glucose transport (Amatruda & Finch,1979). These temperature-dependent alterations in insulin binding and insulin action have been interpreted to result from change in membrane fluidity.

An increase in unsaturated fatty acid content of phospholipids of membranes in erythroleukemia cells (Ginsberg et al.,1981) and Ehrlich ascites cells (Ginsberg et al.,1982) affects the binding parameters of the insulin receptor. In both cases, cells enriched with unsaturated fatty acids exhibit increased insulin binding due to a greater number of available binding sites with a decreased affinity for insulin. Grunfeld et al. (1981) found the 3T3-L1 preadipocytes grown in monounsaturated fatty acid medium, as compared to a saturated fat medium, increased both the affinity and number of insulin receptors and improved insulin mediated glucose transport. Similar effects to those seen in erythroleukemia and Ehrlich ascites cells were observed. In studies with solubilized insulin receptors from turkey erythrocyte membranes incorporated into liposomes of different fatty acid composition (Gould et al.,1982), increased insulin binding and reduced affinity of the receptor were observed in more unsaturated liposomes.

Membrane phospholipid content has also been shown to influence insulin action. Addition of small amount of phosphatidylserine, phosphatidylinositol, cholesterol or sphingomyelin to phospholipid

vesicles of rat adipocyte membrane significantly reduced glucose transport (Sandra et al.,1984; Sandra & Fyler,1982).

Mechanisms by which changes in the lipid component of membranes may influence insulin binding and action have not yet been determined. However,it has been hypothesized that the insulin receptor may exist in two forms, a low affinity form and a high affinity form. According to this model, if the insulin receptor is contained in a saturated, presumably less fluid environment, there may be a greater tendency for the insulin receptor to associate into the high affinity state. Conversely, a more unsaturated environment would allow the insulin receptor to dissociate to the low affinity form which might lead to a greater accessibility of binding sites (Ginsberg et al.,1982).

In rats, changing dietary polyunsaturated to saturated fat ratio (P/S) within the range of that characteristic of the human diet significantly alters membrane fatty acid composition (Field & Clandinin,1989). Feeding a high P/S (2.0) as compared to a low P/S (0.2) diet, significantly increased the polyunsaturated fatty acid composition and decreased the monounsaturated fatty acid composition of the major membrane phospholipids in both control and diabetic animals. The diabetic state was associated with increased linoleic acid and decreased levels of monounsaturated fatty acid and arachidonic acid in most membrane phospholipids. These diabetes associated changes are consistent with previous reports of decreased rates of insulin sensitive hepatic $\Delta 6$ and $\Delta 9$ desaturase enzymes (Faas & Carter,1980).

Diet-induced alterations in membrane phospholipids were associated with changes in insulin receptor function (Field et al.,1988; Field et

al.,1990). Feeding diets with a high P/S ratio as compared to diets with a low P/S ratio, significantly improved insulin binding to adipocytes from normal animals. Scatchard analysis suggested that the increase in binding was due to an increased number of available high affinity low capacity receptor sites. Similarly, the rate of glucose transport and glucose incorporation into lipid was significantly increased in cells from control animals fed a diet high in linoleic acid. While diet fat composition did not affect the amount of insulin bound by cells from diabetic animals, feeding a high linoleic acid diet to diabetic animals significantly improved glucose transport, oxidation and incorporation into lipid (Field et al.,1990).

It has been demonstrated that dietary ω-6 fatty acids are capable of modulating membrane phospholipid composition as well as insulin-mediated functions. It is possible that dietary ω-3 fatty acids may also affect insulin activity in vivo through alterations in membrane microenvironment.

Storlien et al. (1987) have demonstrated that replacement of 6% of the linoleic acid from safflower oil with long-chain ω-3 fatty acids from fish oil prevented development of insulin resistance in skeletal muscle but reduced insulin-stimulated glucose metabolism in white adipose tissue. However, the amount of fat in the diet was exceedingly high at 59% of total kilocalories. It is possible that insulin-stimulated glucose metabolism may have been different at a more physiological diet fat intake.

In contrast, van Amelsvoort et al.(1988) has fed weanling rats semi-synthetic diets with a moderate fat content (34%kcal) from a variety of

oil sources. The sunflower oil diet contained 68% of total lipid as linoleic acid and had a P/S ratio of 5.7 while the linseed oil diet provided 57% linolenic acid and had a P/S ratio of 7.8. Similar results in insulin binding and insulin-stimulated deoxyglucose uptake was demonstrated in adipocytes from animals fed either polyunsaturated fat diet. The inconsistencies noted in these studies appear to be due either to the incorporation of high levels of total fat or total ω-3 fatty acids into the diets fed. Thus, there is a need to investigate the effects of physiological levels of ω-3 fatty acids on insulin binding and action.

E. OMEGA-3 FATTY ACIDS

Biosynthesis

The three major groups of unsaturated fatty acids are the ω-9, ω-6 and ω-3 series. The omega number indicates the position of the first double bond counting from the methyl end of the fatty acid. In the liver, members of the ω-6 and ω-3 series can undergo further desaturation and chain elongation but no interconversion between series can occur in animals. The principal dietary sources of ω-6 fatty acid, linoleic acid, are vegetable oils such as corn and safflower oil. Long chain fatty acids of the ω-3 series (20:5ω3, 22:6ω3) are found in marine fish oils such as mackerel, menhaden, herring and salmon. Eicosapentaenoic acid and docosahexaenoic acid may also be synthesized in vivo from α-linolenic acid (18:3ω3) which is present in linseed oil as well as soybean and canola oils. Members of the ω-3 and ω-6 families are considered essential fatty acids as they cannot be synthesized de novo in animals. Linoleic acid is

an requisite precursor of arachidonic acid (20:4ω6) which in turn is a major component of cell membranes and principal substrate for prostaglandin and leukotriene metabolites. Fatty acids of the ω-3 series are important components of specialized membranes in brain, retinal cells and testis in mammals (as reviewed by Garg et al., 1989). However, the essential functions of tissue ω-3 fatty acids have not been adequately established.

Epidemiologic Studies

Epidemiologic data from Greenland Eskimos, whose diet is highly enriched with ω-3 fatty acids from fish consumption, show a low prevalence of coronary artery disease and non-insulin dependent diabetes mellitus, in spite of a high-fat diet and a tendency towards obesity (Bang & Dyerberg,1985). In Inuits, death from myocardial infarction is rare. A low prevalence of hypertension, a favourable pattern of serum lipids combined with reduced platelet aggregation and a vasodilation are suggested protective mechanisms attributed to shifts in the eicosanoid balance (von Schacky et al.,1987).

Wide interest in using ω-3 fatty acids in diabetic patients has been shown because non-insulin dependent diabetes mellitus is associated with hyperlipoproteinemia, enhanced platelet aggregability and accelerated atherogenesis. As resultant mortality of noninsulin dependent diabetic subjects is twice that of nondiabetic subjects, increased consumption of ω-3 fatty acids may be of benefit.

Omega-3 Fatty Acids in noninsulin dependent diabetes mellitus

To date several studies have examined the short-term effects of ω-3 fatty acids from fish oil in noninsulin dependent diabetic (NIDDM) patients. Some apparently beneficial effects of ω-3 fatty acids on platelet function (Knapp et al.,1986; Haines et al.,1986), eicosanoid formation (von Schacky et al.,1987), plasma triglycerides (Phillipson et el.,1985; Albrink et al.,1986) and blood pressure (Singer et al.,1985; Kasim et al.,1988) have been described. However, controversial data has been reported as to the impact of fish oil on glycemic control, which apparently either improved (Popp-Snijders et al.,1987; Waldhausl et al.,1989) or deteriorated (Friday et al.,1989; Glauber et al.,1988; Schectman et al.,1988) in NIDDM patients. Suggested mechanisms for the changes in glucose metabolism include increased hepatic glucose output, impaired insulin secretion and unchanged glucose disposal rates (Glauber et al.,1988).

Studies in which a deterioration in glycemic control was observed generally supplemented with large doses of fish oil. For example, Schectman et al.(1988) studied 13 NIDDM patients who received 4.0 grams and later 7.5 grams of ω-3 fatty acids for 4 weeks as compared to 12 grams of safflower oil in a single blind crossover design. Other aspects of diet fat intake were not assessed. Compared with the safflower oil treatment, fish oil reduced plasma triglyceride level by approximately 25% at the 4.0 gram dose and approximately 40% at the 7.5 gram dose. In terms of glucose control, at the 7.5 gram dose, fasting glucose and glycosylated hemoglobin levels increased by 20% and 12% respectively, but were unchanged at the 4.0 gram/day level of supplementation. Similarly when

Kasim et al.(1988) supplemented 22 NIDDM patients with 2.8 gram/day of ω-3 fatty acids for 8 weeks, fasting serum glucose level did not change significantly. The mean glycosylated haemoglobin increased significantly after 4 weeks of supplementation and returned to baseline after 8 weeks of supplementation. In a double-blind crossover design study, Borkman et al.(1989) studied 10 individuals with mild NIDDM. Subjects were instructed to supplement their basic diet with 10 grams of fish oil (3 grams ω-3 fatty acid) and 10 grams of safflower oil daily over separate 3 week periods, with an intervening 3 week washout period. Fasting blood glucose increased 14% during fish oil and 11% during safflower oil supplementation compared with baseline values. It has been suggested that the extra dietary energy or fat intake, rather than the ω-3 fatty acids per se may have caused the deterioration in glycemic control observed (Borkman et al.,1989).

These studies demonstrate the difficulty in determining the effect of ω-3 fatty acid in type II diabetic patients. It appears that the doses of ω-3 fatty acids used in patients with diabetes mellitus is extremely important and that harmful effects may be averted at lower doses. Secondly, the subjects studied have varied in their types of medication, degree of obesity and presence or absence of lipid abnormalities, all of which may modify the effect of dietary ω-3 fatty acids. These studies have also not controlled for the fat and calorie content of the fish oils studied and have not conducted long-term studies to determine whether the effects of fish oils are transient or long lasting. More recently, it has also been demonstrated in animals that the balance between dietary saturated and ω-6 polyunsaturated fatty acids is an important factor

determining the efficacy of fish oil in altering biochemical parameters such as plasma cholesterol, triglyceride and arachidonic acid levels (Garg et al.,1988). More studies examining these issues and potential effects on glucose utilization need to be conducted before ω-3 fatty acids can be recommended or discouraged from use by diabetic individuals.

F. INSULIN RESISTANT STATES

Resistance to the action of insulin is central to many disease states such as obesity, insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) in man as well as streptozotocin-induced diabetes in animals. Insulin resistance is defined as a metabolic state in which a normal concentration of insulin produces a less than normal biologic response. While abnormal beta cell secretory function or circulating insulin antagonists may cause insulin resistance, research indicates the primary cause to be a target tissue defect in insulin action (Kolterman et al.,1981). Target tissue defects can be subdivided into receptor and post-receptor (or post-binding) defects.

A biologic dose curve, where a biologic response is measured over a range of insulin concentrations is commonly used to assess insulin action. The predicted consequence of a decrease in the number of receptors would be a rightward shift in the dose-response curve for insulin action. Insulin effect would be decreased at lower insulin concentrations, whereas a normal maximal response would be shown at higher insulin concentrations (Olefsky,1981). As adipocytes possess "spare receptors", a decrease in the number of receptors can only lead to decrease in maximal insulin

action when less than 10% of the original receptor complement is present (Olefsky,1981). A defect in the sequence of insulin action following the initial insulin-receptor binding can also lead to impaired insulin action and insulin resistance. A pure post-receptor defect should lead to reduction in insulin action at all insulin concentrations, which is termed a decrease in insulin responsiveness (Olefsky,1981).

Obesity

Obesity is a well recognized insulin resistant state in man. The insulin resistance is characterized by hyperinsulinemia both in the basal state and after various stimuli of insulin secretion and by decreased biological effects of insulin (Olefsky,1981). The basic alteration(s) responsible for the insulin resistance of target cells in obesity is unknown. Research has focused on either a change in the insulin receptor content or a change in postreceptor events.

Insulin binding to monocytes (Archer et al.,1975; Olefsky,1976), erythrocytes (Kapp & Plotnick,1980) and adipocytes (Olefsky,1976; Livingston et al.,1984; Pedersen et al.,1982) from obese subjects is significantly decreased. Scatchard analysis of these insulin binding data generally show the decrease as a consequence of reduced number of functional receptors rather than a decrease in receptor affinity. While several studies have described an overall decrease in the number of adipocyte insulin receptors per cell between normal and obese individuals (Kolterman et al.,1979a; Olefsky,1976), other work has found only a change in the cell surface concentration of receptors without a change in total receptor number (Livingston et al.,1984; Pedersen et al.,1982). The

larger size of adipocytes from obese individuals as compared to normal subjects can account for a change in the cell surface concentration of the receptor. As the mechanism of coupling between insulin binding and insulin action is unclear, it is not possible to assess whether this dilution effect leads to defective receptor function or signal generation.

It has been hypothesized that the degree of insulin receptor impairment may depend on the severity of obesity and fasting hyperinsulinemia (Pedersen et al.,1982). Pedersen et al.(1982) found a negative correlation between insulin binding and fasting plasma insulin concentration suggesting that in obesity fasting hyperinsulinemia may cause the decrease in receptor number. Chronic hypocaloric dieting normalizes both plasma insulin levels and cellular insulin binding in obese individuals along with some improvement in the in vivo insulin action (Archer et al.,1975; Bar et al.,1976).

Studies of adipocytes from obese subjects demonstrate rightward shifts of the insulin dose response curves for glucose transport, glucose oxidation, lipogenesis and antilipolysis (Pedersen et al.,1982). The authors suggest the decreased insulin sensitivity is most likely the consequence of the impaired insulin binding. However, decreased maximal insulin stimulated glucose transport, glucose oxidation and lipogenesis were also present indicating that a postreceptor defect also exists. These finding are not universal, as other studies have found the maximum response to insulin in cells from obese individuals to be normal for glucose transport (Livingston et al.,1984) and for the antilipolytic effects of insulin (Pedersen et al.,1982; Olefsky, 1977).

Insulin Dependent Diabetes Mellitus

The presence of insulin resistance in type I diabetes mellitus has been confirmed in several studies (Pedersen & Hjollund,1982; Taylor et al.,1984; Yki-Jarvinen et al.,1984; Yki-Jarvinen & Koivisto,1984; Nankervis et al.,1984; Del Prato et al.,1983; Pernet et al.,1984; DeFronzo et al.,1982). Himsworth and Kerr (1939) were among the first to suggest that insulin action may be impaired in insulin dependent diabetes. Using an insulin-glucose tolerance test these researchers found that a significant portion of their insulin dependent subjects were resistant to the action of insulin.

Recent studies have utilized glucose-insulin clamp techniques to measure in vivo glucose metabolism in insulin dependent subjects. Several studies (Nankervis et al.,1984; Yki-Jarvinen & Koivisto,1984; Yki-Jarvinen et al.,1984; Del Prato et al.,1983; DeFronzo et al.,1982) have demonstrated that insulin-mediated glucose metabolism is markedly diminished in insulin dependent diabetics as compared to healthy controls. Basal hepatic glucose production is also significantly elevated in insulin dependent diabetes (DeFronzo et al.,1982; Nankervis et al.,1984; Yki-Jarvinen & Koivisto,1984). However, following hyperinsulinemia hepatic glucose production was similarly suppressed in both diabetic and control subjects indicating normal hepatic sensitivity to insulin (DeFronzo et al.,1982; Nankervis et al.,1984; Yki-Jarvinen & Koivisto,1984). This observation suggests that the primary site of insulin resistance resides in peripheral tissues (DeFronzo et al.,1982).

It has been suggested that insulin therapy aimed at maintaining good diabetic control can ameliorate the insulin resistance of type I diabetics

(Yki-Jarviven & Koivisto,1984). In newly diagnosed type I diabetics, Yki-Jarviven & Koivisto (1984) found that after three months of insulin treatment, insulin sensitivity improved significantly. Similarly, Ginsberg (1977) observed complete normalization of insulin action in newly diagnosed ketotic diabetics after insulin therapy. However, in other studies, only partial improvement (Nankervis et al.,1984) or no improvement (Del Prato et al.,1983) in insulin-mediated action was observed following insulin therapy. Thus, the role of metabolic control in insulin action remains unclear.

Conflicting results have been observed between in vitro insulin binding to adipocytes and blood cell populations. While insulin binding to blood cells has been reported to be normal (Nankervis et al.,1984; Yki-Jarviven et al.,1984; Del Prato et al.,1983; Pedersen & Hjollund,1982), reduced insulin binding to adipocytes from insulin dependent diabetics has been observed (Pedersen & Hjollund,1982; Yki-Jarviven et al.,1984; Taylor et al.,1984). Changes in insulin binding to adipocytes has been attributed to both reductions in receptor number (Yki-Jarviven et al.,1984; Pedersen & Hjollund, 1982) and receptor affinity (Taylor et al.,1984). Pedersen & Hjollund (1982) have hypothesized that relative peripheral hyperinsulinemia produced by subcutaneous insulin therapy may have regulated the number of insulin receptors in adipose tissue.

Adipocytes from insulin dependent diabetic subjects have shown impairments in insulin-mediated function of glucose transport, glucose oxidation, lipogenesis and inhibition of lipolysis as compared to adipocytes from control subjects (Pedersen & Hjollund,1982; Taylor et al.,1984). Decreased insulin binding has been hypothesized to be

responsible for the impaired insulin sensitivity in glucose transport and antilipolysis while a postreceptor defect may be the primary cause for changes in lipogenesis and glucose oxidation (Pedersen & Hjollund,1982). However, more work is necessary to define the insulin resistance characteristic of insulin dependent diabetes mellitus.

Non-Insulin Dependent Diabetes Mellitus

Non-insulin dependent diabetes mellitus (NIDDM) or type II diabetes is a multifactorial disorder characterized by peripheral insulin resistance, increased basal hepatic glucose production and impaired insulin secretion which combine to generate the hyperglycemic state (Olefsky,1985). Within the non-insulin dependent diabetic population, variable degrees of glucose intolerance and impaired insulin secretion and action occur. The relationship between the defective metabolic functions is complex and interdependent.

As the majority of non-insulin dependent diabetics are overweight, obesity is thought to be a contributing factor to the insulin resistance in this disease. However, insulin resistance exhibited by non-insulin dependent diabetics is greater than that due to obesity alone. Additionally, non-obese non-insulin dependent diabetics also demonstrate insulin resistance (Ginsberg & Rayfield,1981). Hollenbeck et al. (1984) examined the relative effects of obesity and NIDDM on <u>in vivo</u> glucose utilization by use of the euglycemic clamp technique. Obese and non-obese diabetic patients had similar plasma glucose levels following ingestion of glucose. While insulin action in NIDDM subjects was markedly reduced as compared to normal controls, the obese condition did not influence

insulin-stimulated utilization of glucose. The researchers concluded that the insulin resistance observed was primarily due to the diabetic condition and obesity appeared to play a minor role.

A number of studies have examined the involvement of an insulin receptor impairment in the etiology of insulin resistance in NIDDM (Kolterman et al.,1981; Mandarino et al.,1984; Lonnroth et al.,1983). Using the euglycemic glucose-clamp technique, Kolterman et al.(1981) investigated the insulin resistance associated with impaired glucose tolerance and type II diabetes. Subjects with impaired glucose tolerance demonstrated a decrease in insulin sensitivity (a rightward shift in the insulin dose-response curve) while maintaining a normal insulin responsiveness. Insulin binding to adipocytes was also decreased on a per cell basis. However, non-insulin dependent diabetic subjects, both obese and non-obese, displayed both a decreased insulin sensitivity and responsiveness (a decrease in maximal rate of glucose disposal) with a similar decline in insulin receptor binding. Furthermore, a negative linear correlation between fasting glucose levels and the maximal glucose utilization rate was found. These investigators concluded that in patients with impaired glucose tolerance, insulin resistance is due to a decreased number of receptors. However, in type II subjects, insulin resistance is due to a decreased number of receptors as well as a post-receptor defect that apparently worsens as the hyperglycemia increases.

Other studies examining the mechanism of insulin resistance in non-insulin dependent diabetes have found decreased insulin sensitivity and unaltered insulin binding in non-insulin dependent diabetics as compared to age and weight-matched control subjects (Mandarino et al.,1984;

Lonnroth et al.,1983). It has been suggested that a defective coupling between insulin binding and insulin action is present in type II diabetes (Mandarino et al.,1984).

The diabetic state has been demonstrated to impair in vitro measures of glucose metabolism such as glucose oxidation (Bolinder et al.,1982), glucose incorporation into lipids (Lonnroth et al.,1983) and glucose transport (Ciaraldi et al.,1982). However, no post-receptor defect has been found to occur in insulin's antilipolytic effect (Bolinder et al., 1982). It has been proposed that the post-receptor defect is specific for one or more aspects of cellular glucose uptake and metabolism (Ciaraldi et al.,1982).

Overproduction of glucose by the liver is also a major contributing abnormality in non-insulin dependent diabetes. Kolterman et al.(1981) found basal rates of hepatic glucose output to be markedly higher in obese and non-obese non-insulin dependent diabetics as compared to control subjects. A close correlation between hepatic glucose production and fasting blood glucose levels was established, indicating that the rate of glucose production by liver directly influences the level of fasting hyperglycemia. While the cause of this increase in hepatic glucose production is not fully understood, hyperglucagonemia is a common feature of non-insulin dependent diabetes (Unger & Orci,1981). It has been shown that approximately two thirds of the hepatic glucose production is glucagon-dependent in normal and non-insulin dependent subjects (Liljenquist et al.,1977; Baron et al.,1984).

It has been demonstrated that treatment which improves glucose tolerance leads to improved insulin secretion and a reduction in insulin

resistance. Hjollund et al.(1987) examined the effect of good glycemic control induced by low-energy dieting in 8 newly diagnosed, untreated non-insulin dependent diabetics. In these subjects, average body weight decreased by 8 kilograms and fasting plasma glucose was also significantly decreased while fasting serum insulin concentrations were unchanged. While adipocyte insulin binding remained unchanged, glucose incorporation into lipids, glucose oxidation and basal and insulin-stimulated glucose transport in adipocytes was significantly increased. The researchers concluded that dietary treatment of moderately obese non-insulin dependent diabetics which leads to good glycemic control results in reversal of the abnormalities observed in adipocytes from untreated NIDDM subjects. Oral sulfonylurea therapy has also been documented to enhance insulin action in adipocytes from NIDDM subjects. Kolterman et al.(1984) found that sulfonylurea therapy increased endogenous insulin secretion, increased binding of insulin to adipocytes after 18 months but not after 3 months of therapy, enhanced peripheral insulin action by acting at a post-receptor site and reduced hepatic glucose output. Similarly, studies examining the effects of insulin therapy have found beneficial results (Scarlett et al.,1982; Andrews et al.,1984; Garvey et al.,1985). In one study, insulin treatment for 2 weeks produced a 72% increase in the maximal rate of insulin-stimulated glucose uptake by peripheral tissues (Scarlett et al.,1982). While insulin binding to adipocytes did not change with therapy, a significant decrease in basal hepatic glucose output was observed.

Streptozotocin Diabetic Rat

Streptozotocin diabetes is one of the best studied models of chemically-induced diabetes. Streptozotocin (2-deoxymethyl-nitrosurea-glucopyranose), a metabolite of the soil organism Streptomyces alcromogenes, causes the selective destruction of the beta cells of pancreatic islets. Once injected, streptozotocin alters the permeability of the plasma membrane of beta cells eliminating the ability to secrete insulin. Thus, streptozotocin causes mild to severe insulin deficiency depending upon the dosage (Bell & Hye,1983).

Adipocytes from streptozotocin diabetic animals respond poorly to insulin; however, the mechanisms of this insulin resistance are not clearly understood. While several studies have found significantly increased insulin binding in adipocytes from streptozotocin-diabetic rats (Schoenle et al.,1977; Kobayashi & Olefsky,1979; Sandra & Fyler,1982), insulin action is markedly diminished. Significantly impaired rates of glucose oxidation (Schoenle et al.,1977; Kobayashi & Olefsky,1979; Sandra & Fyler,1982), glucose incorporation into lipids (Schoenle et al.,1977; Kobayashi & Olefsky,1979) and glucose transport (Schoenle et al.,1977; Kobayashi & Olefsky,1979; Sandra & Fyler,1982) have been reported. Concurrent with insulin-resistant glucose transport activity, streptozotocin diabetes in the rat is associated with a decrease in adipocyte GLUT4 glucose transporter content (Kahn et al.,1989). However, following insulin therapy, adipocyte glucose transport activity is increased 3-fold while GLUT4 transporter content increased only approximately 1.5-fold over control levels. It is hypothesized that insulin treatment of the diabetic rat is also associated with an increase

in glucose transporter intrinsic activity (Kahn & Flier, 1990). Basal glucose transport activity in adipocytes was not significantly altered by diabetes or insulin treatment. Similarly, adipocyte content of GLUT1 transporters was unchanged with diabetes or insulin treatment (Kahn et al., 1989).

Streptozotocin diabetes induces considerable changes in the fatty acid composition of tissues. Monounsaturated fatty acids and arachidonic acid levels are reported to be depressed in liver and adipose tissues (Faas & Carter,1980). These alterations in fatty acid composition may result from depressed $\Delta 6$ and $\Delta 9$ desaturase activities that have been noted in diabetic animals (Faas & Carter,1980). Treatment with insulin only partially normalizes the fatty acid composition, as arachidonic acid levels remain reduced (Faas & Carter,1980).

Diets high in polyunsaturated fatty acids are effective in improving a number of physiological alterations characteristic of streptozotocin diabetes. Improvements have been demonstrated in insulin-stimulated glucose transport and insulin binding in adipocytes from control and diabetic rats fed a high-fat, high P/S diet compared to a high-fat, low P/S diet. These effects coincided with dietary-induced alterations in fatty acyl composition of adipocyte membrane phospholipids. Dietary P/S ratios were significantly related to membrane phospholipid fatty acyl composition. Linear relationships were noted among increased ω-6 fatty acids, decreased total monounsaturated fatty acids and reduced total saturated fatty acids, and dietary fat composition and increased insulin binding affinity (Field et al.,1990). Recent investigations have also demonstrated that a high-fat, high polyunsaturated fat diet significantly

improved plasma and urinary glucose levels in streptozotocin diabetic rats (Rajotte et al.,1988). Dietary manipulation induced a 30% reduction in hemoglobin A1C levels in these animals (Rajotte et al.,1988).

These studies indicate that dietary ω-6 fatty acids are capable of modulating membrane phospholipid composition as well as membrane associated enzyme and receptor functions. It is possible that dietary ω-3 fatty acids may also affect insulin activity through alterations in membrane phospholipid fatty acyl characteristics and membrane microenvironments (Clandinin et al.,1985; Lardinois,1987).

G. RESEARCH PLAN

Rationale

Much recent interest has focused on therapeutic benefits and complications of ω-3 fatty acid supplementation by feeding fish oil. It has been hypothesized that ω-3 fatty acids may improve diabetic glycemic control and blood lipid profiles. However, several studies in NIDDM humans have shown a deterioration in fasting glucose and glycosylated hemoglobin levels following dietary ω-3 fatty acid supplementation suggesting that peripheral glucose utilization may be compromised.

Recent evidence from experimental animals indicates that increasing dietary intake of ω-6 fatty acids while maintaining a constant ω-3 fatty acid intake, increases the ω-6 fatty acid content in adipocyte plasma membrane phospholipids while reducing the levels of ω-3 fatty acids present in these membrane phospholipids. As the insulin receptor is embedded within the lipid bilayer of the plasma membrane, these changes in

membrane lipid composition have been related to improvements in insulin binding and insulin action in both control and diabetic animals. Therefore, it is reasonable to predict that increasing ω-3 fatty acid incorporation into plasma membrane phospholipids will impede insulin binding and action in adipocytes.

Dietary ω-3 fatty acids have dramatic effects on both the polyunsaturated fatty acid composition of membranes and on a variety of functions. However, the relationship among physiological levels of dietary ω-3 fatty acids, adipocyte plasma membrane composition, insulin binding and insulin action has not been clearly addressed. Thus, the objective of this study is to determine the effect of altering dietary ω-3 fatty acids on adipocyte plasma membrane composition, insulin binding and insulin-stimulated glucose transport while maintaining a constant ω-6 fatty acid intake. Recent evidence also suggests that the balance between dietary saturated fatty acids and ω-6 fatty acids is an important factor determining the efficacy of ω-3 fatty acid induced alterations in biochemical parameters. Therefore, in the present study, the effect of dietary ω-3 fatty acid level was examined in animals fed diets with high (Experiment 1) or low (Experiment 2) polyunsaturated to saturated (P/S) fatty acid ratios.

Hypotheses

It is hypothesized that in both normal and diabetic states, a high ω-3 fatty acid intake within a range of intake that is practical for humans will:

1. increase ω-3 fatty acid level in the major adipocyte plasma membrane phospholipids.
2. decrease insulin binding to the adipocyte plasma membrane.
3. decrease insulin-stimulated glucose transport in adipocytes.

These hypotheses will be tested using diets providing either a high (Experiment 1) or low dietary P/S ratio (Experiment 2).

II. MATERIALS AND METHODS

A. ANIMALS AND DIETS

Two experiments were conducted in which identical protocols were followed but differing only in the fat composition of the diets fed. In both experiments, 46 male weanling Sprague-Dawley rats (60 ± 6 grams) were randomly assigned to two groups and fed a semi-purified, high-fat diet containing 27% (w/w) high-protein casein, 38% (w/w) carbohydrate as corn starch and 20% (w/w) fat. The diet was supplemented with the following on a per kilogram basis: 2.75 grams choline, 6.25 grams inositol, 2.5 grams L-methionine, 10 grams vitamin mix (A.O.A.C.), 50 grams Bernhart-Tomerelli mineral mix and 80 grams non-nutritive cellulose. The composition of this basal diet, vitamin and mineral mixes has been reported in detail earlier (Hargreaves & Clandinin, 1987a). The fatty acid composition of the diets are presented in Table II-1. In experiment one, the diet fat composition provided a polyunsaturated to saturated fatty acid ratio (P/S) of 1.9 with the principal difference between the diets being the level of ω-3 fatty acids. The low ω-3 fatty acid diet provided 1.1% (w/w) ω-3 fatty acid as 18:3ω3 from linseed oil while the high ω-3 diet supplied 3.2% (w/w) ω-3 fatty acid as fish oil. In experiment two, the diets provided a P/S ratio of 0.2. The low ω-3 diet contributed 1.0% (w/w) of the diet fat as ω-3 fatty acid from linseed oil and the high ω-3 diet provided 3.1% (w/w) of the diet fat as ω-3 fatty acid from fish oil. All diets were cholesterol-free. Animals were housed individually in a temperature controlled room at 21°±1°C and maintained on a 12 hour light/dark cycle.

TABLE II-1

FATTY ACID COMPOSITION OF DIETS FED

	Experiment #1		Experiment #2	
Dietary ω-3 level:	High	Low	High	Low
	(% w/w of total fatty acids)			
Fatty Acid				
$C_{14:0}$	1.7	1.3	4.3	3.8
$C_{16:0}$	13.5	14.3	28.0	29.6
$C_{16:1}$	1.1	0.2	1.3	0.4
$C_{18:0}$	14.7	14.6	42.8	42.2
$C_{18:1}$	9.2	9.2	3.1	3.1
$C_{18:2(6)}$	54.7	57.7	13.6	16.6
$C_{18:3(3)}$	0.3	1.0	0.1	1.0
$C_{20:0}$	0.1	0.2	0	0.3
$C_{20:5(3)}$	1.8	0	1.9	0
$C_{22:6(3)}$	0.4	0	0.5	0
$\Sigma_{polyunsats}$	57.9	58.8	17.0	17.8
$\Sigma_{(\omega-6)}$	54.8	57.7	13.8	16.8
$\Sigma_{(\omega-3)}$	3.2	1.1	3.1	1.0
Σ_{sats}	30.8	31.2	77.7	78.6
$\Sigma_{monounsats}$	11.3	10.0	5.4	3.6
P/S ratio	1.9	1.9	0.2	0.2

Fatty acid composition of diets as determined by gas liquid chromatography. Abbreviations used: $\Sigma_{polyunsats}$, sum of polyunsaturated fatty acids; $\Sigma_{(\omega-6)}$, sum of (ω-6) unsaturated fatty acids; $\Sigma_{(\omega-3)}$, sum of (ω-3) unsaturated fatty acids; $\Sigma_{monounsats}$, sum of monounsaturated fatty acids; Σ_{sats}, sum of saturated fatty acids; P/S, polyunsaturated to saturated fatty acid ratio.

After three weeks of feeding, diabetes was induced in 13 animals in each diet group by intravenous tail injection of streptozotocin (50 mg/kg body weight; Sigma Chemical Co.,Canada) in an acetate buffer, pH 4.5. Only animals with non-fasting blood glucose levels greater than 300 mg/mL after 14 days were considered diabetic. Animals were fed their respective diet treatment for an additional three weeks.

Each day for 10 days one animal from each diet group was killed by decapitation and blood was collected for plasma glucose and insulin determinations. Serum glucose and insulin was determined using a glucose analyzer (YSI Model 27 Industrial Analyzer, YSI Instrument Co., Yellow Springs, Ohio) and by radioimmunoassay (Insulin RIA 100 kit, Pharmacia Co., Uppsala, Sweden) against a rat insulin standard. Epididymal fat pads were collected and adipocytes were isolated by incubation with a crude collagenase (2.0 mg/g tissue; Cooper Biomedical, Toronto) by a modification of the method of Rodbell (1964). The modification included the use of a glucose-free Krebs-Ringer solution buffered with Hepes (Whitesell & Abumrad,1985) containing 2% (w/v) Fraction V bovine albumin (Sigma Chemicals, Canada).

B. INSULIN BINDING

Isolated adipocytes were incubated in a total volume of 1 mL with ^{125}I-porcine insulin (New England Nuclear, Massachusetts, USA; specific activity of 103 μCi/μg) in the buffer used for cell isolation as described by Olefsky & Reaven (1975). Cells were incubated with labelled and unlabelled insulin at 24°C pH 7.4 for 45 minutes. Insulin binding was

terminated as described by Gammeltoft & Gliemann (1973) by rapidly centrifuging 300 μL aliquots from cell suspensions in polyethylene microcentrifuge tubes to which 100 μL silicon oil had been added (MSE microcentrifuge, John's Scientific, Canada, 570 g X 1 min). The tubes were cut through the silicon oil layer and the cell pellet counted in a Minaxi Auto-Gamma 5000 Series gamma counter (Packard Instrument Co. Inc., Downers Grove, Illinois). Binding was corrected for non-specific binding by incubating cells with ^{125}I-insulin and 20 μg/mL unlabelled insulin. The nonspecific binding was 5-10% of total binding and did not vary with diet treatment or disease state. Cell numbers were determined by using a Coulter ZM counter with a 400 μm aperture (Coulter Electronics, Vancouver) after fixing the cells for 24 hours at 36°C in 2% osmium tetroxide in 0.05 M collidine containing 0.15 M NaCl as described by Hirsch & Gallian (1968). Binding was calculated on a per cell basis and expressed as total amount of insulin bound per 2.0 X 10^5 cells.

C. GLUCOSE TRANSPORT

The nonmetabolizable sugar analogue 3-O-methyl-D-[^{14}C]-glucose was used to measure glucose uptake in fat cells during the initial 5 or 20 seconds by modification of the method described by Whitesell & Abumrad (1985). Adipocytes were suspended in a glucose free buffer (Krebs-Ringer-Hepes) and triplicates of 40 μL were placed in plastic culture tubes. Cells were preincubated without shaking for 30 minutes at 37°C without or with insulin in increasing concentrations. Next, 3-O-methyl-D-[^{14}C]-glucose (0.4 μCi in 10 μL) was added to each tube, swirled and stopped

after 5 seconds for insulin stimulated transport or 20 seconds for basal (no insulin) transport with 300 μL cold phloretin (0.3 mM in albumin-free Krebs-Ringer Hepes buffer). Cell suspensions were transferred to 500 μL polyethylene microtubes containing 100 μL silicon oil and rapidly centrifuged in a MSE microcentrifuge (John's Scientific, Canada) on low speed (6500 rpm X 45 seconds). The time between stopping the reaction and separating cells was less than 3 minutes. The tubes were cut through the silicon oil layer and the cell pellet counted in 5 mL Bio-HP (Fisher Scientific, Canada) in a Beckman LS-5801 scintillation counter at a counting efficiency of approximately 97%. All samples were done in triplicate and corrected for extracelluar trapped radioactivity. Extracelluar trapped radioactivity was measured by adding tracer to cells suspended in 300 μL phloretin. In both experiments, glucose transport was measured over a range of insulin concentrations (0-1000 ng/mL) at a glucose concentration of 0.5 mM. Cell numbers were determined as described above by (Hirsch & Gallian,1968). Glucose transport was calculated on a per cell basis and expressed as nmoles transported/5 sec/2.0 X 10^5 cells.

D. PLASMA MEMBRANE ISOLATION

Plasma membranes were prepared from adipocytes according to the method of Lewis et al. (1979) modified to allow isolation of plasma membranes from small 1 mL samples of material using a Beckman TL-100 ultracentrifuge (Beckman Instruments Ltd., Palo Alto, California). The modification included loading the second pellet, resuspended in 200 μL buffer (0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl) at pH 7.4, on an 800 μL

linear gradient containing 32-52% (w/v) sucrose, 1 mM EDTA and 10 mM Tris-HCl, pH 7.4, followed by centrifugation at 45,000 rpm (205,780 X g at r_{ave}) in a Beckman TLS-55 rotor for 30 minutes at 4°C. The white band near the top of the gradient containing the plasma membrane fraction was collected and diluted to a volume of 1 mL in 10mM Tris-HCl, 1 mM EDTA buffer (pH 7.4) and centrifuged at 30,000 rpm (30,000 X g at r_{ave}) for 10 minutes at 4°C in a Beckman TL-100 rotor. The membrane pellet was collected, resuspended in 400 μL 10 mM Tris-HCl buffer and stored at -70°C for analysis of lipid composition. The purity of this plasma membrane fraction has been established previously by assaying 5'nucleotidase and succinate dehydrogenase (Field et al.,1990).

E. MEMBRANE LIPID ANALYSIS

Lipids were extracted from plasma membranes by a modified Folch procedure (Folch et al.,1957). This extraction procedure was modified as follows: to 400 μL Tris-HCl buffer pH 7.4 containing the membrane pellet, 0.8 mL methanol, 2.0 mL chloroform:methanol (1:1 v/v), 2.7 mL chloroform and 2.5 mL chloroform:methanol (2:1 v/v) were added in succession. The layers were split by addition of 1.6 mL of 0.1 mM KCl. The lower phase containing phospholipids was collected. Individual phospholipids were separated on Whatman HP-K thin layer chromatography plates (10 X 10 cm) using the following solvent system: chloroform:propionic acid:1-propanol:water (20:20:30:10 by volume) (Brasitus et al.,1986). Separated phospholipids were sprayed with 0.03% w/v 2'7'dichlorofluorescein in 0.01 M NaOH and detected by comparison under u.v. light with appropriate standards.

Fatty acid methyl esters from phosphatidylcholine, phophatidylethanolamine, phosphatidylserine, phosphatidylinositol and sphingomyelin were prepared using 14% w/v BF_3/methanol reagent (Morrison & Smith,1964) and separated by automated gas-liquid chromatography (Vista 6000 GLC and Vista 402 data system; Varian Instruments, Georgetown, Ontario, Canada). Chromatography was performed using a fused silica BP20 capillary column (25m X 0.25mm I.D.; Varian, Georgetown, Ontario, Canada). Helium was used as the carrier gas at a flow rate of 1.8 mL/min using a splitless injection mode. The initial oven temperature was 150°C, increased to 190°C at 20°C/min and held for 23 minutes, then increased to 220°C at 2°C/min for a total analysis time of 40 minutes. The analytical conditions used separate all saturated, mono-, di-, and polyunsaturated fatty acid from C_{14} to C_{24} carbons in chain length.

F. STATISTICAL ANALYSIS

Significant effects of diet treatment and disease state on glucose transport and insulin binding were determined by two-way analysis of variance procedures over the insulin concentrations used. Membrane phospholipid fatty acid composition, animal weights, serum insulin and glucose levels were compared by analysis of variance procedures and significant effects further defined by Duncan's multiple range test (Steel & Torrie,1980). Except for the sum of monounsaturated fatty acid in the phosphatidylethanolamine fraction of experiment 2, no significant interaction of diet and disease state was found. All data is expressed as mean ± standard error (SEM).

III. RESULTS

A. ANIMAL WEIGHTS AND SERUM GLUCOSE AND INSULIN LEVELS

In both experiment 1(high P/S) and experiment 2(low P/S), control animals weighed significantly more than diabetic animals (Table III-1). In experiment 1, control animals fed a low ω-3 fatty acid diet exhibited higher final body weights than control animals fed a high ω-3 fatty acid diet. Diet treatment did not affect serum glucose or insulin levels in either experiment. Postprandial serum glucose levels were significantly higher in all diabetic animals than control animals. Serum insulin levels were significantly lower in all diabetic groups compared to control animals.

B. INSULIN BINDING

The total amount of specific insulin bound expressed per 2.0 X 10^5 adipocytes from experiment 1 and 2 is presented in Figure III-1 and Figure III-2, respectively. In experiment 1, at insulin concentration of 0.1 ng/mL, adipocytes from control animals fed the high ω-3 fatty acid diet bound significantly more ($p<0.05$) insulin than adipocytes from control animals fed the low ω-3 fatty acid diet or from either diabetic group. When analyzed across the eight insulin concentrations measured, cells from control animals bound significantly more ($p<0.025$) insulin than cells from diabetic animals. This difference in insulin binding became more pronounced at higher insulin concentrations (Figure III-1).

TABLE III-1

ANIMAL WEIGHTS, SERUM GLUCOSE AND INSULIN LEVELS

Treatment	Diet	(n)	Final Body Weight (g)	Final serum levels of Glucose (mg/dL)	Insulin (μU/mL)
Experiment #1 (P/S 1.9)					
Control	high ω-3	(10)	373 ± 7[b]	136 ± 5[a]	57 ± 10[a]
Animals	low ω-3	(10)	404 ± 11[a]	142 ± 5[a]	83 ± 20[a]
Diabetic	high ω-3	(13)	317 ± 10[c]	502 ± 40[b]	21 ± 4[b]
Animals	low ω-3	(13)	325 ± 12[c]	419 ± 52[b]	29 ± 9[b]
Experiment #2 (P/S 0.2)					
Control	high ω-3	(10)	367 ± 6[a]	133 ± 4[a]	69 ± 5[a]
Animals	low ω-3	(10)	348 ± 7[a]	134 ± 2[a]	54 ± 8[a]
Diabetic	high ω-3	(13)	275 ± 5[b]	587 ± 24[b]	21 ± 5[b]
Animals	low ω-3	(13)	258 ± 10[b]	538 ± 33[b]	20 ± 4[b]

Within an experiment, values (mean±SEM) within a column without a common superscript are significantly different ($p<0.05$).

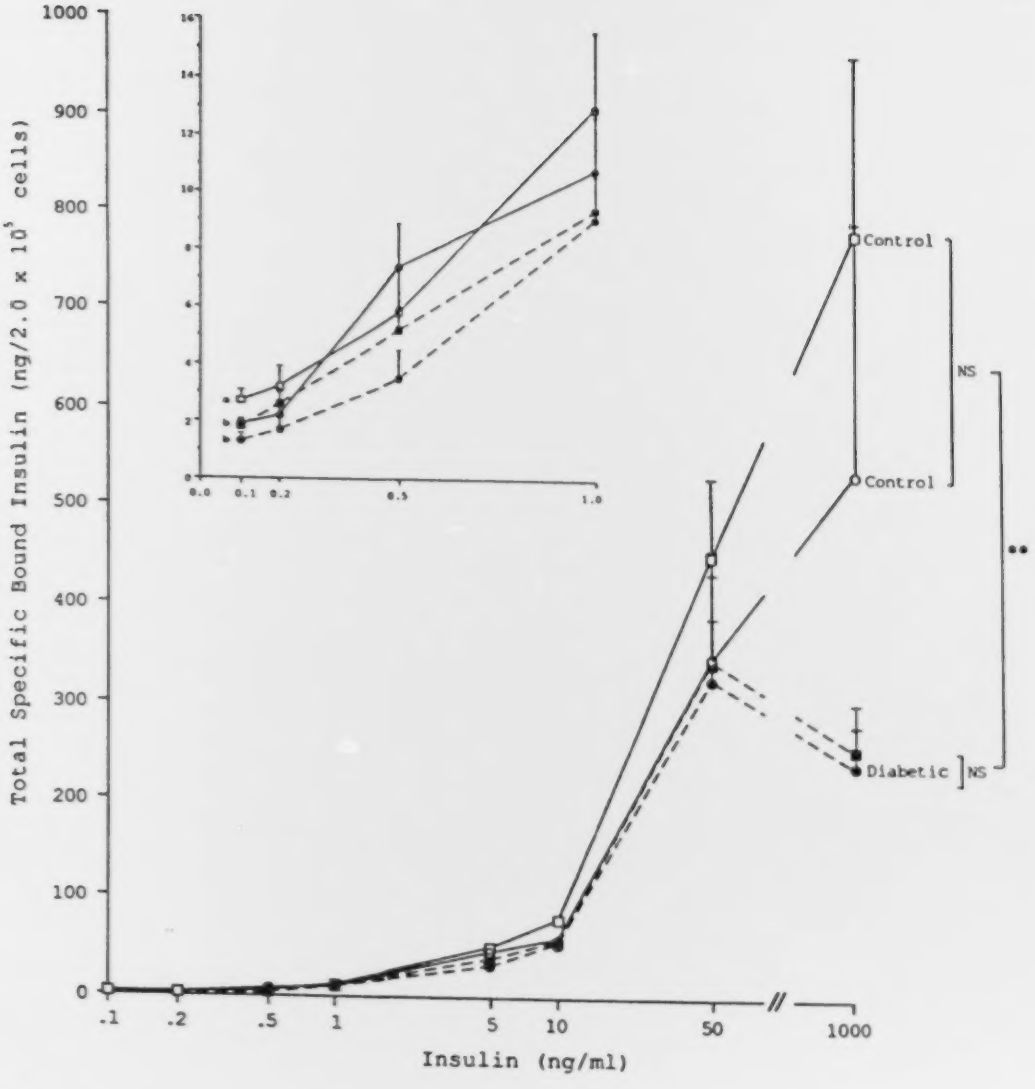

Figure III-1. Experiment 1 (P/S 1.9) Total specific bound insulin at 24°C to adipocytes.

Total specific insulin bound at each insulin concentration are group means±SEM for 8 control animals fed the high ω-3 diet (□—□); 8 control animals fed the low ω-3 diet (o—o); 10 diabetic animals fed the high ω-3 diet (■—■); and 8 diabetic animals fed the low ω-3 diet (●—●). Groups were compared by analysis of variance calculated over 8 insulin concentrations tested (••, $p<0.025$).
Insert shows an expansion of graph at lower insulin levels. Binding at 0.1 ng/mL insulin level was compared by analysis of variance. Values without a common superscript are significantly different ($p<0.05$).

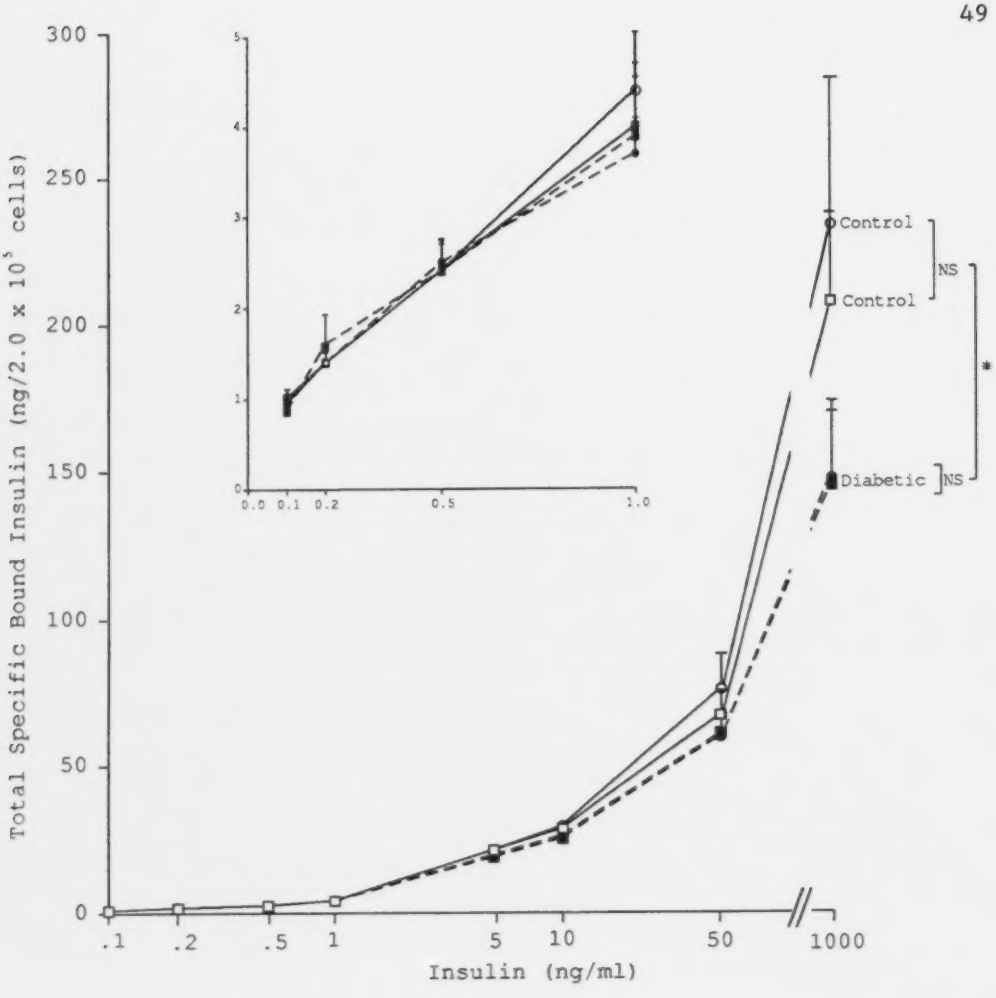

Figure III-2. Experiment 2 (P/S 0.2) Total specific bound insulin at 24°C to adipocytes.

Total specific insulin bound at each insulin concentration are group means±SEM for 10 control animals fed the high ω-3 diet (□—□); 10 control animals fed the low ω-3 diet (o—o); 11 diabetic animals fed the high ω-3 diet (■---■); and 11 diabetic animals fed the low ω-3 diet (●---●). Groups were compared by analysis of variance calculated over 8 insulin concentrations tested (*, $p<0.05$). Insert shows an expansion of graph at lower insulin levels.

In experiment 2, an effect of diet on insulin binding was not observed in either control or diabetic adipocytes (Figure III-2). When analyzed across the eight insulin concentrations measured using repeated measures ANOVA, cells from control animals bound significantly more ($p<0.05$) insulin than cells from diabetic animals.

C. GLUCOSE TRANSPORT

Insulin stimulated glucose transport observed in experiment 1 and 2 are presented in Figures III-3 and III-4, respectively. In experiment 1, diet treatment did not significantly affect glucose transport in cells from control or diabetic animals. Adipocytes from diabetic animals fed either diet treatment transported significantly less glucose ($p<0.001$) than cells from control animals.

In experiment 2, within cells from control or diabetic animals, diet treatment did not significantly affect glucose transport. Compared to adipocytes from control animals, diabetic cells transported significantly less glucose ($p<0.001$) over the higher insulin concentrations (25 ng/mL - 1000 ng/mL).

D. EFFECT OF DIET AND THE DIABETIC STATE ON THE FATTY ACID COMPOSITION OF ADIPOCYTE MEMBRANE PHOSPHOLIPIDS

Both ω-3 fatty acid content of the diet and diabetic state significantly altered the fatty acid composition of major membrane phospholipids of the adipocyte plasma membrane. The predominant fatty acids within each phospholipid class are illustrated in Tables III-2 to

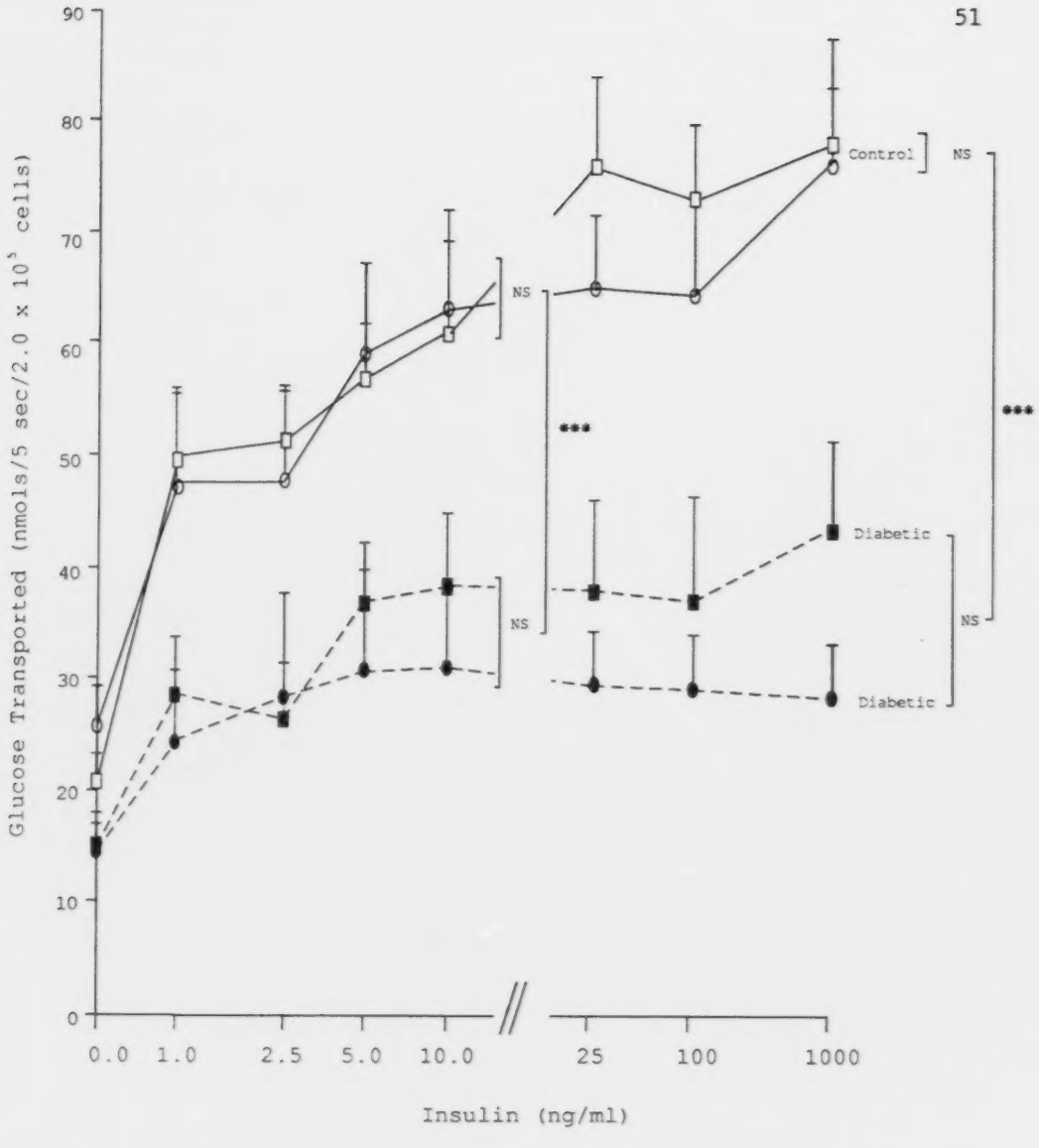

Figure III-3. Experiment 1 (P/S 1.9) Insulin stimulated glucose transport.

The amount of glucose transported at 5mM glucose are group means±SEM for 9 control animals fed the high ω-3 diet (□—□); 8 control animals fed the low ω-3 diet (o—o); 11 diabetic animals fed the high ω-3 diet (■---■); and 10 diabetic animals fed the low ω-3 diet (●---●). Statistics were conducted across the lower insulin concentrations (0-10 ng/mL) and the higher insulin concentrations (25, 100, 1000 ng/mL). The level of significance is indicated (***, $p<0.001$).

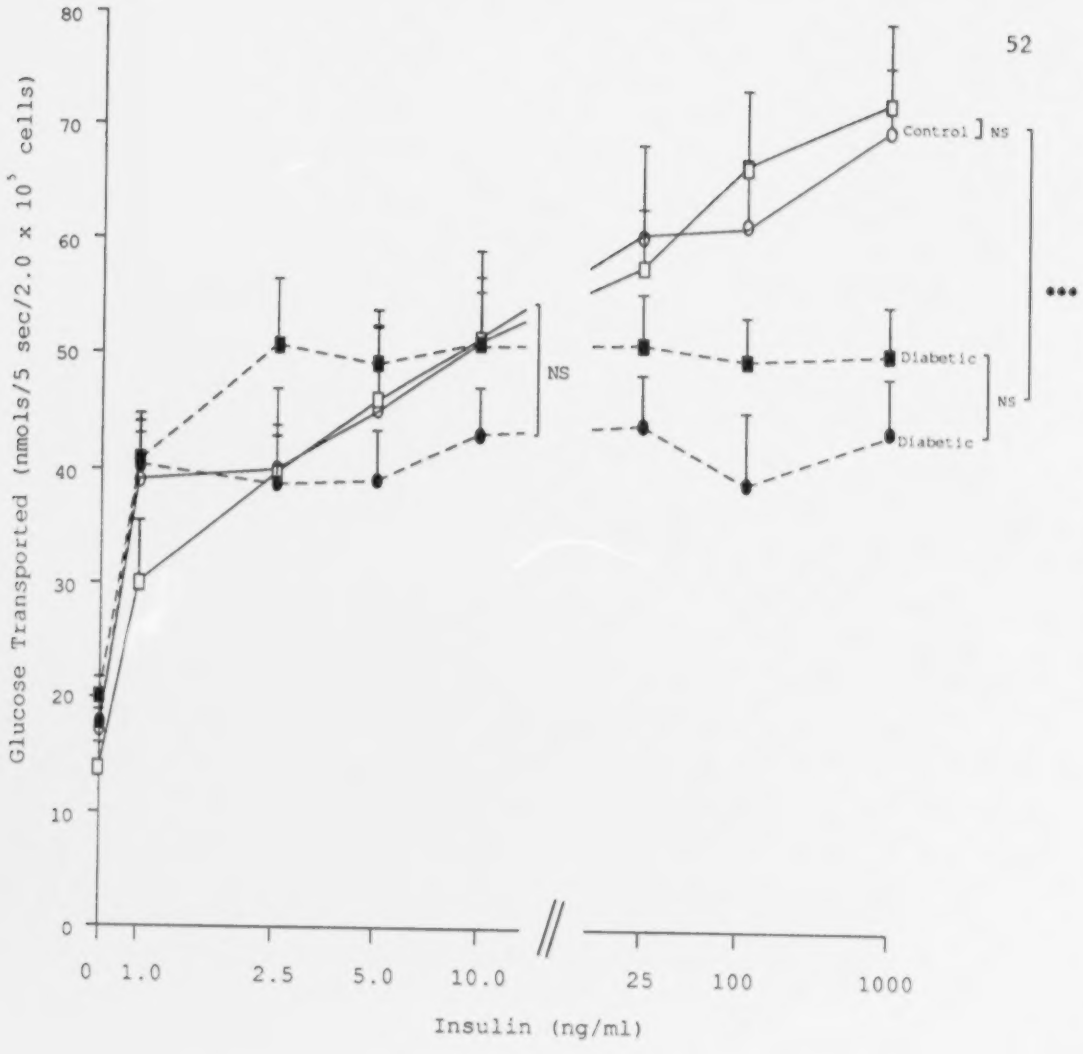

Figure III-4. Experiment 2 (P/S 0.2) Insulin stimulated glucose transport.

The amount of glucose transported at 5mM glucose are group means±SEM for 10 control animals fed the high ω-3 diet (□—□); 9 control animals fed the low ω-3 diet (o—o); 13 diabetic animals fed the high ω-3 diet (■---■); and 10 diabetic animals fed the low ω-3 diet (●---●). Statistics were conducted across the lower insulin concentrations (0-10 ng/mL) and the higher insulin concentrations (25, 100, 1000 ng/mL). The level of significance is indicated (***, $p<0.001$).

III-6.

Phosphatidylcholine

Altering the ω-3 fatty acid level of a high P/S diet did not produce significant change in fatty acyl composition of phosphatidylcholine from control or diabetic animals (Table III-2). The diabetic state was not associated with alteration in fatty acyl composition of either high P/S diet. In experiment 2, increased dietary ω-3 fatty acid level was associated with a significantly decreased arachidonic acid ($C_{20:4(6)}$) content and increased ω-3 fatty acid (eicosapentaenoic acid ($C_{20:5(3)}$), docosahexaenoic acid ($C_{22:6(3)}$) and total ω-3 fatty acid) content of phosphatidylcholine in adipocyte membranes from both control and diabetic animals. Compared to diet-matched control adipocytes, diabetic cells from animals fed the low ω-3 diet had significantly lower oleic acid ($C_{18:1(9)}$) content in phosphatidylcholine.

Phosphatidylethanolamine

In experiment 1,increased ω-3 fatty acid level of the diet significantly increased the $C_{20:5(3)}$,$C_{22:6(3)}$ and total ω-3 fatty acid content of phosphatidlyethanolamine from control membranes (Table III-3). Diet treatment also increased the $C_{20:5(3)}$, $C_{22:6(3)}$ and total ω-3 fatty acid content of phosphatidylethanolamine from diabetic membranes, but these changes did not reach statistical significance. In experiment 1, the diabetic state was not associated with significant fatty acid modification of adipocyte membrane phosphatidylethanolamine.

TABLE III-2

EFFECT OF DIET AND THE DIABETIC STATE ON THE FATTY ACID COMPOSITION OF PHOSPHATIDYLCHOLINE

Composition (% w/w)

Dietary fatty acid ω-3 level:	Experiment #1 (P/S 1.9)					Experiment #2 (P/S 0.2)				
	High		Low		Significance	High		Low		Significance
Disease:	C	D	C	D		C	D	C	D	
Fatty Acid										
C16:0	21.3±1.6	21.1±2.4	22.7±2.7	23.2±2.6	NS	20.8±2.6	20.7±1.5	21.4±0.9	21.6±0.8	NS
C18:0	35.7±1.1	37.0±2.2	38.0±3.8	34.4±1.5	NS	30.4±0.9	29.1±1.1	28.4±0.4	30.1±1.1	NS
C18:1(9)	5.9±0.7	7.2±1.9	4.7±0.7	5.7±1.1	NS	$8.6±0.2^{a}$	$7.4±0.3^{ab}$	$8.3±0.3^{a}$	$6.9±0.6^{b}$	0.05
C18:2(6)	22.5±1.5	20.8±3.4	16.3±4.5	20.3±3.6	NS	23.6±1.0	25.7±1.0	22.7±1.1	23.5±1.7	NS
C20:4(6)	7.8±0.7	6.4±1.6	9.4±2.4	7.7±1.3	NS	$4.9±1.4^{a}$	$4.7±0.5^{a}$	$10.9±0.5^{b}$	$8.8±0.7^{b}$	0.001
C20:5(3)	0.3±0.1	0.1±0.1	0.3±0.2	0.3±0.1	NS	$1.9±0.5^{a}$	$1.5±0.3^{a}$	$0.1±0.1^{b}$	$0.1±0.1^{b}$	0.001
C22:6(3)	1.4±0.6	0.6±0.3	0.6±0.3	0.3±0.1	NS	$2.6±0.5^{a}$	$1.3±0.5^{a}$	$0.5±0.1^{b}$	$0.4±0.2^{b}$	0.001
ΣSFA	58.1±1.1	60.2±5.2	61.8±6.5	59.3±4.5	NS	53.9±2.5	53.3±1.7	53.2±0.5	55.2±1.4	NS
ΣMUFA	8.5±0.8	9.7±2.1	8.4±0.6	8.8±1.5	NS	11.8±0.5	12.1±1.1	11.6±0.8	9.7±1.0	NS
Σω-6	31.4±1.1	28.9±4.7	28.1±6.9	31.1±4.6	NS	$29.0±1.8^{a}$	$31.0±1.1^{ab}$	$33.7±0.8^{ab}$	$34.3±2.2^{b}$	0.05
Σω-3	1.9±0.7	1.3±0.3	1.8±0.7	0.9±0.3	NS	$5.4±1.2^{a}$	$3.6±1.0^{a}$	$0.7±0.2^{b}$	$0.8±0.4^{b}$	0.001

Only fatty acids affected by diet manipulation are reported (mean ± S.E.). Significant effects by two-way analysis of variance procedures; values without a common superscript are significantly different. Abbreviations used: C, control; D, diabetic; ΣSFA, sum of saturated fatty acids; ΣMUFA, sum of monounsaturated fatty acids; Σ(ω-6), sum of ω-6 fatty acids; Σ(ω-3), sum of ω-3 fatty acids.

TABLE III-3

EFFECT OF DIET AND THE DIABETIC STATE ON THE FATTY ACID COMPOSITION OF PHOSPHATIDYLETHANOLAMINE

Composition (% w/w)

Dietary fatty acid ω-3 level:	Experiment #1 (P/S 1.9)					Experiment #2 (P/S 0.2)				
	High		Low		Significance	High		Low		Significance
Disease:	C	D	C	D		C	D	C	D	
Fatty Acid										
C16:0	20.2±2.7	17.0±3.4	14.8±3.6	15.6±2.9	NS	12.2±2.2	10.3±0.9	11.1±2.7	14.5±2.9	NS
C18:0	32.6±2.7	29.6±0.5	31.6±1.5	29.3±1.8	NS	24.7±1.8	26.4±1.3	26.8±1.2	26.1±2.0	NS
C18:1(9)	7.7±1.4	10.8±1.3	7.8±0.6	9.7±1.6	NS	7.9±0.5	7.9±0.3	8.9±0.4	8.4±0.4	NS
C18:2(6)	17.9±3.3	14.2±2.1	14.0±2.4	14.5±2.2	NS	15.4±1.0	18.3±1.0	15.6±1.4	13.0±2.4	NS
C20:4(6)	13.6±1.0	14.7±3.5	20.7±2.9	16.0±2.8	NS	$12.7±1.4^{a}$	$14.3±0.7^{a}$	$25.2±2.7^{b}$	$19.1±3.8^{ab}$	0.01
C20:5(3)	$0.8±0.3^{a}$	$0.4±0.2^{ab}$	$0.3±0.1^{b}$	$0.2±0.1^{b}$	0.05	$5.5±0.5^{a}$	$5.0±0.6^{a}$	$0.2±0.1^{b}$	$0.2±0.2^{b}$	0.001
C22:6(3)	$4.8±1.3^{a}$	$2.4±0.4^{b}$	$1.7±0.5^{b}$	$1.1±0.4^{b}$	0.05	$7.3±1.3^{a}$	$5.8±1.0^{a}$	$1.3±0.2^{b}$	$1.2±0.2^{b}$	0.001
ΣSFA	53.9±5.4	48.7±4.2	47.8±5.4	47.2±5.4	NS	41.1±3.7	41.1±2.3	41.0±4.6	46.7±5.0	NS
ΣMUFA	11.1±2.6	15.5±1.8	10.6±0.4	15.3±3.0	NS	$12.8±0.4^{ab}$	$11.7±0.4^{b}$	$12.4±0.5^{ab}$	$13.5±0.6^{a}$	0.05
Σω-6	27.7±2.7	31.9±5.4	39.3±4.9	35.2±5.2	NS	$30.5±2.1^{a}$	$34.5±1.3^{ab}$	$43.8±4.1^{b}$	$37.1±5.2^{ab}$	0.05
Σω-3	$7.3±1.3^{a}$	$3.9±1.0^{b}$	$2.3±0.6^{b}$	$2.2±0.5^{b}$	0.01	$15.6±2.5^{a}$	$13.3±2.0^{a}$	$2.9±0.5^{b}$	$2.7±0.4^{b}$	0.001

Only fatty acids affected by diet manipulation are reported (mean ± S.E.). Significant effects by two-way analysis of variance procedures; values without a common superscript are significantly different. Abbreviations used: C, control; D, diabetic; ΣSFA, sum of saturated fatty acids; ΣMUFA, sum of monounsaturated fatty acids; Σ(ω-6), sum of ω-6 fatty acids; Σ(ω-3), sum of ω-3 fatty acids.

In experiment 2, increased dietary ω-3 fatty acid level significantly decreased the $C_{20:4(6)}$ content and the total ω-6 fatty acid content of phosphatidylethanolamine in control membranes. Concurrently, the ω-3 fatty acid content ($C_{20:5(3)}$, $C_{22:6(3)}$ and total ω-3 fatty acid) increased in both adipocyte membranes from control and diabetic animals. The total monounsaturated fatty acid content of phosphatidylethanolamine was associated with a significant interaction ($p<0.05$) of dietary ω-3 fatty acid level and diabetes.

Phosphatidylinositol

The ω-3 fatty acid level and diabetic state did not significantly modify the fatty acyl composition of adipocyte membrane phosphatidylinositol from animals fed either high P/S diet (Table III-4).

An elevated ω-3 fatty acid level in the low P/S diet significantly decreased the $C_{20:4(6)}$ content and significantly increased the $C_{20:5(3)}$ and total ω-3 fatty acid content of phosphatidylinositol in control membranes. In diabetic animals, increased ω-3 fatty acid level of the diet significantly increased the $C_{20:5(3)}$ content. Compared to diet-matched control animals, diabetic animals fed a low ω-3 fatty acid diet displayed increased $C_{16:0}$ content in membrane phosphatidylinositol.

Phosphatidylserine

In experiment 1, altering the dietary ω-3 fatty acid level was not associated with any significant changes in fatty acyl composition of phosphatidylserine from control animal membranes (Table III-5). However, elevated dietary ω-3 fatty acid level significantly increased the total

TABLE III-4

EFFECT OF DIET AND THE DIABETIC STATE ON THE FATTY ACID COMPOSITION OF PHOSPHATIDYLINOSITOL

Composition (% w/w)

Dietary fatty acid ω-3 level:	Experiment #1 (P/S 1.9)					Experiment #2 (P/S 0.2)				
	High		Low		Significance	High		Low		Significance
Disease:	C	D	C	D		C	D	C	D	
Fatty Acid										
C16:0	19.2±3.7	25.0±5.9	20.6±6.2	26.7±5.0	NS	9.0±2.8^{ab}	13.4±2.1^{ab}	8.0±1.4^{b}	14.3±1.6^{a}	0.05
C18:0	45.2±3.3	34.4±1.5	39.4±3.5	37.1±2.8	NS	34.8±3.5	41.1±1.8	41.3±1.6	40.4±1.8	NS
C18:1(9)	6.8±2.7	10.6±3.3	5.4±0.8	7.4±2.5	NS	4.7±0.7	3.7±0.3	4.1±0.5	4.4±0.5	NS
C18:2(6)	6.6±2.0	8.5±3.7	6.6±1.7	6.3±1.1	NS	7.7±3.5	6.4±1.5	5.0±0.6	4.3±1.0	NS
C20:4(6)	12.8±2.4	7.8±3.9	12.9±3.8	9.5±3.4	NS	15.8±2.9^{a}	18.0±1.6^{a}	30.3±3.9^{b}	18.5±2.6^{a}	0.05
C20:5(3)	0.2±0.1	0.4±0.2	0.2±0.1	0.4±0.1	NS	3.6±1.4^{a}	3.4±0.5^{a}	0.4±0.1^{b}	1.4±0.4^{b}	0.01
C22:6(3)	0.7±0.2	0.9±0.5	0.4±0.2	0.8±0.3	NS	2.5±0.7	2.0±0.5	1.3±0.5	1.2±0.7	NS
ΣSFA	66.8±8.1	61.6±8.3	62.2±9.4	66.8±4.3	NS	52.5±3.8	59.6±2.3	54.2±2.8	61.1±3.8	NS
ΣMUFA	10.3±3.1	16.9±2.9	12.8±2.7	12.1±2.8	NS	12.3±1.4^{a}	6.8±0.7^{c}	7.0±1.1^{bc}	10.3±1.2^{ab}	0.01
Σω-6	21.3±5.1	19.7±5.6	21.9±5.9	19.4±4.2	NS	26.1±2.1	26.4±1.7	36.5±4.0	24.9±3.3	NS
Σω-3	1.7±0.7	1.8±0.5	0.8±0.3	1.7±0.4	NS	9.0±2.2^{a}	7.1±1.3^{ab}	2.3±0.4^{c}	3.7±1.0^{bc}	0.01

Only fatty acids affected by diet manipulation are reported (mean ± S.E.). Significant effects by two-way analysis of variance procedures; values without a common superscript are significantly different. Abbreviations used: C, control; D, diabetic; ΣSFA, sum of saturated fatty acids; ΣMUFA, sum of monounsaturated fatty acids; Σ(ω-6), sum of ω-6 fatty acids; Σ(ω-3), sum of ω-3 fatty acids.

TABLE III-5

EFFECT OF DIET AND THE DIABETIC STATE ON THE FATTY ACID COMPOSITION OF PHOSPHATIDYLSERINE

Composition (% w/w)

Dietary fatty acid ω-3 level:	Experiment #1 (P/S 1.9)					Experiment #2 (P/S 0.2)				
	High		Low		Significance	High		Low		Significance
Disease:	C	D	C	D		C	D	C	D	
Fatty Acid										
C16:0	20.7±2.4	22.2±4.1	18.7±3.9	18.4±5.4	NS	8.3±1.2	7.8±1.2	6.9±1.9	12.1±2.1	NS
C18:0	43.0±2.7	38.8±2.4	43.8±2.5	42.3±2.4	NS	41.8±1.7	42.1±1.2	41.5±1.8	42.3±0.6	NS
C18:1(9)	8.1±1.4	8.1±1.8	5.9±1.2	7.7±2.2	NS	$6.8±0.4^{a}$	$5.2±0.6^{bc}$	$6.5±0.2^{ab}$	$5.0±0.6^{c}$	0.01
C18:2(6)	9.5±2.3	10.2±3.1	11.2±2.5	13.0±3.3	NS	19.2±1.6	20.6±1.1	19.5±2.3	14.0±2.7	NS
C20:4(6)	4.4±1.5	5.0±1.9	8.5±1.9	8.2±2.8	NS	$5.6±0.7^{a}$	$6.2±0.5^{ab}$	$13.0±1.7^{c}$	$9.7±1.7^{bc}$	0.001
C20:5(3)	0.4±0.2	1.4±0.9	0.4±0.2	0.1±0.1	NS	$2.2±0.2^{a}$	$2.0±0.3^{a}$	$0.2±0.1^{b}$	$0.9±0.4^{b}$	0.001
C22:6(3)	1.2±0.6	2.7±1.2	0.6±0.2	0.4±0.1	NS	$4.8±0.6^{a}$	$2.8±0.6^{b}$	$0.5±0.2^{c}$	$0.6±0.1^{c}$	0.001
ΣSFA	65.9±3.8	63.6±4.6	64.5±5.4	62.6±5.2	NS	$53.8±2.7^{b}$	$55.6±1.7^{ab}$	$51.8±1.4^{b}$	$62.3±3.7^{a}$	0.05
ΣMUFA	12.1±1.3	12.4±2.8	9.2±1.5	10.9±3.0	NS	10.8±0.7	8.9±1.1	9.5±0.7	9.2±1.0	NS
Σω-6	19.0±3.5	18.7±4.4	24.2±4.3	25.4±5.9	NS	25.9±2.4	28.3±0.7	35.0±3.7	25.4±3.8	NS
Σω-3	$3.1±0.8^{ab}$	$5.3±2.2^{a}$	$2.1±0.6^{ab}$	$1.0±0.2^{b}$	0.05	$9.5±1.3^{a}$	$7.1±1.3^{a}$	$1.7±0.3^{b}$	$2.6±0.6^{b}$	0.001

Only fatty acids affected by diet manipulation are reported (mean ± S.E.). Significant effects by two-way analysis of variance procedures; values without a common superscript are significantly different. Abbreviations used: C, control; D, diabetic; ΣSFA, sum of saturated fatty acids; ΣMUFA, sum of monounsaturated fatty acids; Σ(ω-6), sum of ω-6 fatty acids; Σ(ω-3), sum of ω-3 fatty acids.

ω-3 fatty acid content in phosphatidylserine isolated from diabetic adipocyte membranes. The diabetic state was not associated with significant alterations in the fatty acid composition of phosphatidylserine for experiment 1.

In experiment 2, increased dietary ω-3 fatty acid level significantly decreased the $C_{20:4(6)}$ content in control membranes and significantly increased the ω-3 fatty acid ($C_{20:5(3)}$, $C_{22:6(3)}$ and total ω-3 fatty acid) content in both control and diabetic states. The diabetic state was associated with a significantly lower level of $C_{18:1(9)}$ in membrane phosphatidylserine. Compared to diet-matched control adipocytes, diabetic cells from animals fed the high ω-3 fatty acid diet had a significantly lower content of $C_{22:6(3)}$ in phosphatidylserine. Diabetic animals fed the low ω-3 fatty acid diet exhibited a significantly higher level of total saturated fatty acids compared with control animals fed a similar diet.

Sphingomyelin

The ω-3 fatty acid level and diabetic state did not significantly alter the fatty acyl composition of sphingomyelin from animals fed a high P/S diet (Table III-6). However, the diabetic animals fed a high ω-3 fatty acid, low P/S diet exhibited significantly lower levels of $C_{20:0}$ and $C_{20:1}$ fatty acids than diet-matched control animals.

TABLE III-6

EFFECT OF DIET AND THE DIABETIC STATE ON THE FATTY ACID COMPOSITION OF SPHINGOMYLELIN

Composition (% w/w)

Dietary fatty acid ω-3 level:	Experiment #1 (P/S 1.9)					Experiment #2 (P/S 0.2)				
	High		Low		Significance	High		Low		Significance
Disease:	C	D	C	D		C	D	C	D	
Fatty Acid										
C16:0	41.7±4.3	40.7±4.6	43.4±2.5	33.9±2.0	NS	37.2±4.1	42.2±3.0	45.3±3.1	36.2±3.8	NS
C18:0	29.1±4.4	22.5±5.9	28.9±6.5	21.6±2.5	NS	25.9±1.2	27.6±1.1	25.9±2.5	27.3±0.8	NS
C18:1(9)	3.9±1.9	5.6±2.4	3.2±1.1	9.0±3.6	NS	1.8±0.4	1.7±0.1	0.9±0.2	7.6±3.6	NS
C18:2(6)	1.4±0.7	1.3±0.8	1.3±0.6	3.2±1.0	NS	0.9±0.4	0.8±0.3	0.5±0.2	1.4±0.3	NS
C20:4(6)	1.2±0.1	3.6±1.4	1.8±1.1	3.1±1.4	NS	1.6±0.7	1.8±0.6	0.7±0.5	2.6±1.1	NS
C20:0	2.4±1.1	2.6±0.5	3.0±1.2	2.2±0.9	NS	$4.9±0.9^{a}$	$2.4±0.7^{b}$	$4.6±1.0^{ab}$	$3.0±0.3^{ab}$	0.05
C20:1	2.9±1.2	1.2±0.5	2.9±1.7	1.4±0.8	NS	$5.8±1.2^{a}$	$2.6±0.3^{b}$	$3.4±0.7^{b}$	$1.9±0.6^{b}$	0.05
ΣSFA	81.1±6.6	73.6±3.6	80.8±5.1	67.5±4.4	NS	81.6±4.1	87.3±1.9	89.9±1.4	79.3±3.9	NS
ΣMUFA	13.1±5.0	15.9±4.4	12.1±4.8	19.3±3.2	NS	12.1±3.0	7.6±1.0	6.7±0.4	15.8±6.6	NS
Σω-6	4.8±1.5	6.8±1.7	5.5±1.4	10.0±2.3	NS	4.5±0.8	4.2±0.7	3.9±0.7	5.9±1.1	NS
Σω-3	1.0±0.2	3.8±1.7	1.7±0.6	3.2±1.4	NS	1.8±1.0	0.9±0.4	0.7±0.2	1.6±0.2	NS

Only fatty acids affected by diet manipulation are reported (mean ± S.E.). Significant effects by two-way analysis of variance procedures; values without a common superscript are significantly different. Abbreviations used: C, control; D, diabetic; ΣSFA, sum of saturated fatty acids; ΣMUFA, sum of monounsaturated fatty acids; Σ(ω-6), sum of ω-6 fatty acids; Σ(ω-3), sum of ω-3 fatty acids.

IV. DISCUSSION

It was hypothesized that in both normal and diabetic states, a high ω-3 fatty acid intake will increase the ω-3 fatty acid level in the major adipocyte plasma membrane phospholipids. Within a high P/S diet, increasing the ω-3 fatty acid content of the diet was found to produce modest increases in both ω-3 fatty acid content of adipocyte plasma membrane phosphatidylethanolamine in control animals (Table III-3) and adipocyte plasma membrane phosphatidylserine in diabetic animals (Table III-5). However, increasing the ω-3 fatty acid content of a low P/S diet produces significant increases in plasma membrane phospholipid ω-3 fatty acid content in both control and diabetic animals (Table III-2 to Table III-5). Concurrently, significant reductions occurred in the arachidonic acid content of the major adipocyte membrane phospholipids from control animals (Table III-2 to Table III-5) and in membrane phosphatidylcholine from diabetic animals (Table III-2). These observations are consistent with previous reports that the balance between dietary saturated and ω-6 fatty acid influences ω-3 fatty acid levels in serum and tissues (Garg et al.,1988). Eicosapentaenoic acid has a greater affinity than arachidonic acid for acyltransferase (Yamashita et al.,1973), thus incorporation of eicosapentaenoic acid into membrane phospholipids is favoured. Additionally, in liver microsomes, ω-3 fatty acids inhibit Δ6 and Δ5 desaturase activity (Garg et al.,1988a; Garg et al.,1988b). Thus, when ω-3 fatty acids are fed in association with high levels of ω-6 fatty acids (linoleic acid), linoleic acid tends to increase arachidonic acid production while ω-3 fatty acids tend to decrease arachidonic acid

production. The competition that exists when ω-3 fatty acids are fed in association with high levels of ω-6 fatty acids results in no change or only a small change in arachidonic acid levels (Table III-2 to Table III-5). However, when ω-3 fatty acids are fed in combination with a high saturated fat level, less competition exists between ω-3 and ω-6 fatty acids, leading to a greater incorporation of ω-3 fatty acids into membrane phospholipids (Table III-2 to Table III-5).

Individual phospholipid classes respond to dietary fatty acid manipulations to different degrees. The molecular mechanisms through which dietary fat composition influences the fatty acid composition of membrane phospholipid are not fully understood. Rates of de novo synthesis of phospholipid (Hargreaves & Clandinin,1987), redistribution of fatty acid chains via phospholipase (Van den Bosch,1980) or acyl transferase (Hargreaves & Clandinin,1987a) and direct desaturation of membrane phospholipid-linked fatty acids (Garg et al.,1988a) have been demonstrated to be altered by dietary fat composition. Similarly, the availability of fatty acids at specific sites of synthesis may be a factor. Adipose tissue fat composition is reflective of the dietary fatty acid composition (Field & Clandinin,1984) and represents a large pool of available fatty acids for synthesis of membrane phospholipids (Field et al.,1988).

Streptozotocin-induced diabetes has been demonstrated to induce considerable change in the fatty acid composition of tissues. Arachidonic acid and monounsaturated fatty acid levels are reported to be depressed in liver and adipose tissues. These changes have been correlated with decreased activity of insulin dependent microsomal Δ6 and Δ9 desaturation

in liver (Faas & Carter,1980). In the present study, diabetic animals fed a low P/S, low ω-3 fatty acid diet had a significantly reduced arachidonic acid level in phosphatidylinositol compared to diet-matched control animals. Similarly, while not significant, there was a tendency towards reduced arachidonic acid levels in other adipocyte plasma membrane phospholipid fractions from diabetic animals fed a low ω-3 fatty acid diet of either high or low P/S type. The diabetic state did not further reduce arachidonic acid levels in membrane phospholipids induced by a high ω-3 fatty acid diet.

Significantly depressed oleic acid levels in phosphatidylcholine and phosphatidylserine and a significantly increased palmitic acid level in phosphatidylinositol were observed in membrane phospholipids from diabetic animals fed a low P/S, low ω-3 fatty acid diet. While these results do not illustrate the prominent decline in monounsaturated fatty acids reported by Faas & Carter (1980) and Worcester et al. (1979) in chow fed animals, these observations parallel membrane changes reported by Field et al. (1988) for animals fed similar semipurified high fat diets.

Secondly, it was hypothesized that a high ω-3 fatty acid content of the diet would be associated with a decrease in insulin binding to the adipocyte plasma membrane. In control animals, a high P/S, high ω-3 fatty acid diet was associated with improved insulin binding at the 0.1 ng/mL insulin concentration. Otherwise, alterations in the ω-3 fatty acid level of the diet did not influence insulin binding within control or diabetic groups.

Previous studies utilizing adipocytes from animals fed high or low P/S diets established that adipocytes from control animals fed a high P/S

diet displayed improved insulin binding (Field et al.,1988). Similarly, in vitro cell cultures enriched with unsaturated fatty acids exhibited increased insulin binding due to increased number of available binding sites with a decreased affinity for insulin (Ginsberg et al.,1982; Ginsberg et al.,1981). In the present study, the total content of polyunsaturated fatty acids present in the adipocyte plasma membrane was unchanged by dietary supplementation of ω-3 fatty acids (Tables III-2 to III-5). This suggests that the amount of polyunsaturated fatty acids present in the membrane, regardless of ω-6 or ω-3 fatty acid type, is a more important determinant of insulin binding. This finding is similar to previous work by van Amelsvoort et al. (1988) who found comparable insulin binding in adipocytes from rats fed diets where the fat component was primarily linoleic acid or linolenic acid.

Mechanisms by which a more polyunsaturated membrane environment may influence insulin binding have not yet been determined. However, it has been hypothesized that a polyunsaturated membrane may increase the accessibility of the receptor for its ligand (Gould et al.,1982) or alter the structure of the receptor (Ginsberg et al.,1981) thus increasing insulin binding.

In both of the present experiments, adipocytes from diabetic animals consistently bound less insulin than adipocytes from control animals. Previous studies have demonstrated increased insulin binding from streptozotocin diabetic rats fed high-carbohydrate, low-fat diets (Schoenle et al.,1977; Kobayashi & Olefsky,1979; Sandra & Flyer,1982). These findings are consistent with the concept that low circulating insulin levels will up-regulate insulin receptors (Gavin et al.,1974).

However, adipocytes from high-fat, low-carbohydrate fed diabetic rats have been shown to bind less insulin than control adipocytes (Field et al.,1988). It has been suggested that feeding a high-fat, low-carbohydrate diet may alter the comparison between insulin binding in diabetic and control animals (Field et al.,1988). High-fat diets may impair the ability to reinsert receptors into the plasma membrane following insulin-induced internalization (Begum et al.,1985).

Next, it was hypothesized that a high ω-3 fatty acid level in the diet would be associated with a decrease in insulin-stimulated glucose transport in adipocytes. In the present study, altering the ω-3 fatty acid content of a high or low P/S diet did not significantly affect the rate of glucose transport in adipocytes within control or diabetic groups (Figures III-1 & III-2).

At all insulin concentrations in experiment 1 and at the higher insulin concentrations in experiment 2, diabetic cells clearly demonstrated insulin-resistance by transporting significantly less glucose as compared to control cells. Significantly impaired rates of glucose transport have previously been reported from adipocytes of streptozotocin-induced diabetic rats (Schoenle et al.,1977; Kobayashi & Olefsky,1979; Sandra & Flyer,1982). However, the mechanisms of insulin resistance in streptozotocin diabetic animals are not clearly understood. The reduced insulin-stimulated glucose transport activity has been associated with a decrease in adipocyte GLUT4 glucose transporter content (Kahn et al., 1989).

In the second experiment, the amount of glucose transported at the lower insulin concentrations did not differ between cells from control and

diabetic animals. This suggests that a high saturated fat diet decreased the amount of glucose transported in control cells but before this can be concluded, an intergrated experiment should be repeated including at least one of the diets from experiment 1 to allow for appropriate statistical analysis. In vitro studies have indicated that insulin-stimulated glucose transport is decreased in a more saturated lipid environment (Sandra & Flyer,1982; Pilch et al.,1980). Similarly, adipocytes from control and diabetic animals fed low P/S diets transported significantly less glucose as compared to cells from animals fed a high P/S diet (Field et al.,1990).

From this study, it can be concluded that while moderate dietary supplementation of ω-3 fatty acids (3% of total diet fat) in diets of high or low P/S types increased levels of ω-3 fatty acids in plasma membrane phospholipids, these membrane changes were not associated with diminished insulin binding and glucose transport in adipocytes from control or diabetic animals.

Varying the dietary ω-3 fatty acid content of the diet fat produced significant changes in adipocyte plasma membrane phospholipid composition in control and diabetic animals. These changes were particularly evident in low P/S diets. Future studies should examine the effect of these changes on the composition and functioning of prostaglandins, leukotrienes and phosphatidylinositol and its phosphorylated species.

While the membrane changes produced in this study did not affect insulin binding or glucose transport in adipocytes, dietary ω-3 fatty acids may affect glucose utilization in other insulin-sensitive tissues such as muscle or liver. Future studies to determine the effects of

dietary ω-3 fatty acids on glycemic control should address whole body *in vivo* glucose utilization through use of glucose clamp or minimal model techniques. Additionally, dietary ω-3 fatty acid supplementation may influence long-term functions of insulin such as nuclear binding and subsequent nucleic acid and protein synthesis. Thus, before ω-3 fatty acid supplements should be prescribed as safe for use by diabetics in terms of glycemic control, further studies are necessary in other tissues.

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